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Drinking Water Criteria Document for Acrylamide (Final Draft)

Life Systems, Inc., Cleveland, OH

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The Office of Drinking Water (ODW), U.S. Environmental Protection Agency has prepared a Drinking Water Criteria Document on acrylamide.
This Criteria Document is an extensive review of the following topics:

- Physical and chemical properties of acrylamide
- Toxicokinetics and human exposure to acrylamide
- Health Effects of acrylamide in humans and animals
- Mechanisms of toxicity of acrylamide
- Quantification of toxicological effects of acrylamide

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TABLE OF CONTENTS

PAGE

LIST OF FIGURES iii

LIST OF TABLES iv

I. SUMMARY I-1

II. PHYSICAL AND CHEMICAL PROPERTIES II-1

- A. Physical and Chemical Properties II-1
- B. Manufacture and Uses II-4
- C. Summary II-7

III. TOXICOKINETICS III-1

- A. Absorption III-1
- B. Distribution III-1
- C. Metabolism III-6
- D. Excretion III-8
- E. Bioaccumulation and Retention III-11
- F. Summary III-17

IV. HUMAN EXPOSURE IV-1

V. HEALTH EFFECTS IN ANIMALS V-1

- A. General Toxicity V-1
  - 1. Lethality V-1
  - 2. General Effects V-5
- B. Target Organ Toxicity V-12
  - 1. Acute Exposure V-12
  - 2. Short-Term Exposure V-16
  - 3. Longer Term Exposure V-26
- C. Other Effects V-43
  - 1. Carcinogenicity V-43
  - 2. Mutagenicity V-51
  - 3. Reproductive/Teratogenic Effects V-53
- D. Summary V-58

continued-

Table of Contents - continued

PAGE

VI. Health Effects in Humans VI-1

A. Clinical Case Studies VI-1
B. Epidemiologic Studies VI-6
C. High Risk Subpopulations VI-7
D. Summary VI-7

VII. MECHANISM OF TOXICITY VII-1

A. Effects on Enzyme Activities VII-1
B. Effects on Protein Synthesis VII-5
C. Effects on Axonal Transport VII-7
D. Effects on Neurotransmitters and Receptors VII-10
E. Synergistic/Antagonistic Effects VII-18
F. Summary VII-23

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS VIII-1

A. Non-Carcinogenic Effects. VIII-5

1. Lethality VIII-5
2. Short-Term Exposure VIII-6
3. Longer-Term Exposure VIII-11

B. Quantification of Non-Carcinogenic Effects VIII-23

1. One-Day Health Advisory VIII-23
2. Ten-Day Health Advisory VIII-25
3. Adjusted Acceptable Daily Intake VIII-27

C. Carcinogenic Effects VIII-31
D. Quantification of Carcinogenic Risk VIII-33
E. Existing Guidelines and Standards VIII-34
F. Special Considerations VIII-34

1. High Risk Subpopulations VIII-34
2. Interactions VIII-34

G. Summary VIII-35

IX. REFERENCES IX-1

LIST OF FIGURES

FIGURE		PAGE
II-1	Flowsheet for Production of Acrylamide	II-5
III-1	Excretion of (^{14}C) in Urine Following a Single Intravenous Dose of 1-^{14}C-Acrylamide (100 mg/kg)	III-9
III-2	Expiration of $^{14}CO_2$ After a Single Intravenous Dose of (1-^{14}C) Acrylamide (100 mg/kg)	III-12
III-3	Elimination of Parent Acrylamide from Tissues After 10 mg/kg iv	III-13
III-4	Tissue Distribution of Radiolabel Following 10 mg/kg Acrylamide Administered iv	III-15
III-5	Blood and Plasma Levels of Radiolabel After 10 mg/kg Acrylamide Administered iv	III-16
V-1	Effect of Acrylamide Administered Orally on Development of Lung Tumors in A/J Mice	V-50

LIST OF TABLES

TABLE		PAGE
II-1	Physical Properties of Acrylamide	II-2
III-1	Distribution of (^{14}C) in Tissues After a Single Intravenous Dose of (1-^{14}C) Acrylamide (100 mg/kg)	III-2
III-2	Distribution of ^{14}C in Tissues Following 13 Daily Oral Doses of 0.05 mg/kg or 30 mg/kg of 1,3-^{14}C-Acrylamide	III-4
III-3	Concentrations of Non-protein Sulfhydryl in Tissues After A Single Oral Dose of Acrylamide	III-7
V-1	Summary of Lethality Studies of Single Dose Oral Exposure to Acrylamide	V-3
V-2	Summary of Lethality Following Multiple Exposures to Acrylamide	V-6
V-3	Body and Tissue Weights, and Cholesterol and Phospholipid Concentration in Plasma, Red Blood Cell, Liver and Epididymal Fat Pad	V-10
V-4	Summary of General Effects of Acrylamide	V-13
V-5	Summary of Acrylamide Effects Following Short-Term Exposure	V-27
V-6	Summary of Acrylamide Effects Following Long-Term Exposure	V-44
V-7	Tumor-Bearing Animals and Classification of Histologically Examined Skin Tumors in Sencar Mice Initiated with Acrylamide by Various Routes	V-48
V-8	Effect of Acrylamide and Analogs on the Body and Testicular Weights in Mice, and the Effect of Metabolic Activation	V-59
VII-1	Effect of Acrylamide and Diethyl Maleate (DEM) on Brain Neurotransmitter Concentrations	VII-12
VII-2	Effect of Acrylamide (50 mg/kg) Treatment in Rats on Regional NA, DA and 5-HT Concentrations of Brain	VII-14
VII-3	Pharmacological Agents Used in Cerebral Receptor Studies	VII-15
VII-4	Effect of a Single Dose of Acrylamide on Cerebral Receptors	VII-16

continued-

List of Tables - continued

TABLE		PAGE
VII-5	Summary of Neurotoxicity of Compounds and of Their Additive Effects with Acrylamide	VII-20
VIII-1	Comparison of Values from Candidate Studies Identifying Non-Carcinogenic End-Points of Toxicity	VIII-32
VIII-2	Summary of Quantification of Toxicological Effects of Acrylamide	VIII-36

I. SUMMARY

Acrylamide (CH_2=$CHCONH_2$) is a white crystalline solid which is readily soluble in water. The vinyl group of the molecule is electrophilic and readily undergoes attack by a variety of nucleophiles. One form of this reaction is polymerization and this is employed to produce a spectrum of products that have widespread use in industry and science.

Acrylamide is rapidly and completely absorbed from the gastrointestinal tract. It quickly distributes throughout the body with no marked preferential accumulation, except in red blood cells. The majority of total acrylamide present in tissues is bound to protein. The principal metabolic fate of acrylamide is conjugation with cellular glutathione. Exposure to acrylamide results in decreased cellular glutathione levels and excretion in the urine of the mercapturic acid derived from the acrylamide-glutathione adduct. In rats, urine is the principal avenue of excretion: over 60% of a dose of acrylamide (either oral or parenteral) appears in the urine, mostly within 24 hours. About 5% to 6% of a dose is excreted in the feces, and about 6% of a dose undergoes oxidation of the amide carbon with release of CO_2.

Free (parent) acrylamide is rapidly cleared from the body, being undetectable by 24 hours. The metabolites and adducts of acrylamide are cleared more slowly, with biological half-lives up to eight days in some tissues. Thus, there is considerable retention of acrylamide in tissues, and this may underlie the cumulative toxicity properties of acrylamide (as described later).

Acrylamide is highly toxic to animals. The LD_{50} observed in mice, rats, guinea pigs and rabbits for a single oral dose is between 110 and 280 mg/kg and the LD_{50} observed in rats and mice following multiple exposures is from 15 to 80 mg/kg/day, varying inversely with exposure duration.

Most adverse health effects of acrylamide in animals result from neurotoxic effects. Both central and peripheral effects are observed, with sensory neurons being more sensitive than motor neurons. The most characteristic effects are ataxia and weakness in hind limbs, but these external signs are usually preceded by histological or biochemical evidence of nerve damage.

The doses (both oral and parenteral) required to produce signs of neurotoxicity depend inversely on exposure period. Following administration of a single dose of acrylamide to rats, 40 mg/kg produces a decrease in axonal transport, while 15 mg/kg does not. When multiple doses are administered over one to four weeks, doses of 5 to 30 mg/kg/day produce effects. When the exposure period is several months to one year, doses as low as 1 mg/kg/day have been found to produce effects.

Recent evidence indicates that acrylamide may be a carcinogen in animals following both oral and parenteral exposures. Female rats receiving 2 mg/kg/day for 18 months displayed increased frequency of several tumor types. Male rats receiving a dose of 0.5 mg/kg/day for two years had an increased frequency of scrotal tumors, and animals receiving a dose of 2 mg/kg/day for two years had an increased frequency of scrotal, thyroid and adrenal tumors. Mice receiving average doses of 5.4 to 21.4 mg/kg/day for two weeks had increased tumor production in response to the phorbol ester, 12-0-tetradecanoyl-phorbol-13-acetate (TPA). Mice receiving 2.7 to 10.7 mg/kg/day for eight weeks had increased incidence of lung adenomas.

Acrylamide did not produce mutagenic activity in the Ames test (with or without metabolic activation) or in the hepatocyte primary culture DNA repair assay. Chromosome aberrations were observed in spermatogonia from mice receiving 75 mg/kg/day for two to three weeks, but marrow cells were not affected. A striking decrease in mitotic index was observed in both marrow cells and spermatogonia in mice following a single dose of 50 to 150 mg/kg.

Acrylamide freely crosses the placenta in pregnant females, but doses of 20 to 40 mg/kg/day administered to rats during gestation did not cause obvious adverse effects on the pups. More sensitive tests indicate there are effects of acrylamide, both prenatal and lactational, on enzyme levels and neurotransmitter binding in pups.

A number of cases of acrylamide toxicity in humans have been described. The pattern of toxic effects is quite similar to that exhibited in animals and is also dependent on the concentration and duration of exposure. Early signs of human toxicity include tenderness in fingers, numbness in lower limbs and weakness in hands and feet. Longer exposures lead to loss of proprioceptive sensations, decreased reflexes, ataxia, gait disorder and muscle atrophy.

Most cases of human exposure involve workers exposed under occupational circumstances where quantitative information on doses is lacking. In one case, a family was exposed to acrylamide by ingestion and use of well water containing 400 mg/L of acrylamide. This exposure (estimated to be about 11.4 mg/kg/day for 70-kg adult) lasted approximately one month and resulted in symptoms as described above which were serious enough to require hospitalization. Recovery was complete within two to four months after exposure ceased.

The mechanism of acrylamide toxicity is unknown. Most studies have focused on the neurotoxicity of acrylamide and a number of actions which may contribute to this effect have been identified, including: (1) inhibition of certain key enzymes, especially those of energy metabolism; (2) inhibition of protein synthesis; (3) decreased axonal transport and (4) decreased levels of neurotransmitters and/or receptors. The role which these effects play in the appearance of neuropathology remains to be clarified.

A number of chemicals influence acrylamide toxicity. Diethyl maleate increases toxicity, apparently by reducing cellular glutathione levels. Vitamin B_6 supplementation results in a modest reduction in acrylamide toxicity by one test but not by another. Several derivatives of acrylamide, which are themselves neurotoxic, increase neurotoxicity when administered with acrylamide, but it is not clear if the effects are additive or synergistic. Pretreatment of rats with chemicals which induce hepatic microsomal enzymes resulted in increased acrylamide toxicity in one case but not in another.

The most reliable and sensitive estimate available in the literature of a no-observed-adverse-effect level (NOAEL) following a single dose of acrylamide is 15 mg/kg (observed in rats). Although this is derived from a study employing intraperitoneal injection, it is considered acceptable for calculation of an oral risk factor because acrylamide is rapidly and completely absorbed by any route of exposure. Using this NOAEL, values for the one-day Health Advisories (HAs) in children and adults were calculated to be 1.5 mg/L and 5.25 mg/L, respectively. The most appropriate estimate available in the literature of a NOAEL for a ten-day exposure is 3 mg/kg/day in rats (although the experimental period was actually 21 days). Using this NOAEL, values for ten-day HAs in children and adults were calculated to be 0.3 mg/L and 1.05 mg/L, respectively. The most appropriate long-term exposure NOAEL available in the literature (derived from a 90-day study) is 0.2 mg/kg/day in rats. Using this NOAEL, an Acceptable Daily Intake (ADI) of 0.014 mg/day and an Adjusted Acceptable Daily Intake (AADI) of 0.007 mg/L were calculated.

Although acrylamide appears to be carcinogenic in animals, there are insufficient data to permit calculation of excess human cancer risk factors.

The American Conference of Governmental Industrial Hygienists in 1968 recommended and adopted an occupational time-weighted exposure standard for acrylamide of 0.3 mg/m^3. This value was also recommended by the National Institute for Occupational Safety and Health in 1976. No other standards for acrylamide exposure were located in the available literature.

II. PHYSICAL AND CHEMICAL PROPERTIES

A. Physical and Chemical Properties

Acrylamide (CH_2=$CHCONH_2$), also called 2-propenamide or acrylicamide, is a white crystalline solid which is highly soluble in water and moderately soluble in several organic solvents (Bikales and Kolodny 1963, MacWilliams 1978). Some important physical properties of acrylamide are presented in Table II-1.

The acrylamide molecule consists of two functional groups: an amide group and a vinyl group that is conjugated with it. Both groups undergo numerous chemical reactions. Typical examples of the chemical reactions of the amide group are:

(1) Hydrolysis with either acid or base to form acrylic acid:

$$CH_2{=}CHCONH_2 \xrightarrow{H^+ \text{ or } OH^-} CH_2{=}CHCOOH + NH_3$$

(2) Reaction with strong dehydrating agents like phosphorous pentoxide to yield acrylonitrile:

$$CH_2{=}CHCONH_2 \xrightarrow{P_2O_5} CH_2{=}CHCN + H_2O$$

(3) Reaction with formaldehyde under basic conditions to form an alcohol, or under acidic conditions to form N,N'-methylene bis-acrylamide:

Table II-1 Physical Properties of Acrylamide

Property	Value
Molecular weight	71.08
Melting point (°C)	84.5±0.3
Boiling point (°C)	
2 mm Hg	87
5 mm Hg	103
25 mm Hg	125
Vapor Pressure (mm Hg)	
25°C	0.007
40°C	0.033
50°C	0.070
Density (g/mL at 30°C)	1.122
Solubility (g/100 mL at 30°C)	
Water	215.5
Methanol	155.0
Ethanol	86.2
Acetone	63.1
Ethyl acetate	12.6
Chloroform	2.66
Benzene	0.346
n-Heptane	0.0068

Adapted from MacWilliams 1978.

$$CH_2{=}CHCONH_2 \xrightarrow[HCHO]{base} CH_2{=}CHCONHCH_2OH$$

$$2CH_2{=}CHCONH_2 \xrightarrow[HCHO]{acid} (CH_2{=}CHCONH)_2CH_2 + H_2O$$

Typical reactions of the double bond of the vinyl group are:

(1) Addition of halogen to the double bond of acrylamide; this reaction is quantitative in the case of bromine and may be used as a method of analysis. Chlorine (e.g., in treated water) would react similarly.

$$CH_2{=}CHCONH_2 + Br_2 \longrightarrow CH_2BrCHBrCONH_2$$

(2) Addition of nucleophiles to the double bond. The ability of the amide group to withdraw electrons facilitates attack by nucleophilic reagents and this reaction proceeds easily. Most aliphatic amines and ammonia can be added to the double bond without a catalyst. Addition of mercaptans, including hydrogen sulfide, alcohols and ketones, requires a strong base as a catalyst. Since the reaction introduces the 2-carbamoylethyl group into the substrate, the reaction is often called carbamoylethylation.

$$RNH_2 + CH_2{=}CHCONH_2 \longrightarrow RNHCH_2CH_2CONH_2$$

$$RSH + CH_2{=}CHCONH_2 \xrightarrow{C_2H_5ONa} RSCH_2CH_2CONH_2$$

B. Manufacture and Uses

While some miscellaneous methods of preparing acrylamide are of historic interest, only production from acrylonitrile is commercially important (Bikales and Kolodny 1963). Acrylonitrile is particularly suitable as starting material because of its low cost and ready conversion to acrylamide sulfate by treatment with sulfuric acid:

$$CH_2{=}CHCN + H_2SO_4 \xrightarrow{H_2O} CH_2{=}CHCONH_3^+ \cdot HSO_4^-$$

(acrylonitrile) (acrylamide sulfate)

Another production method involves conversion of acrylonitrile to 3-chloropropionamide with hydrochloric acid which, upon treatment with alkali, yields acrylamide:

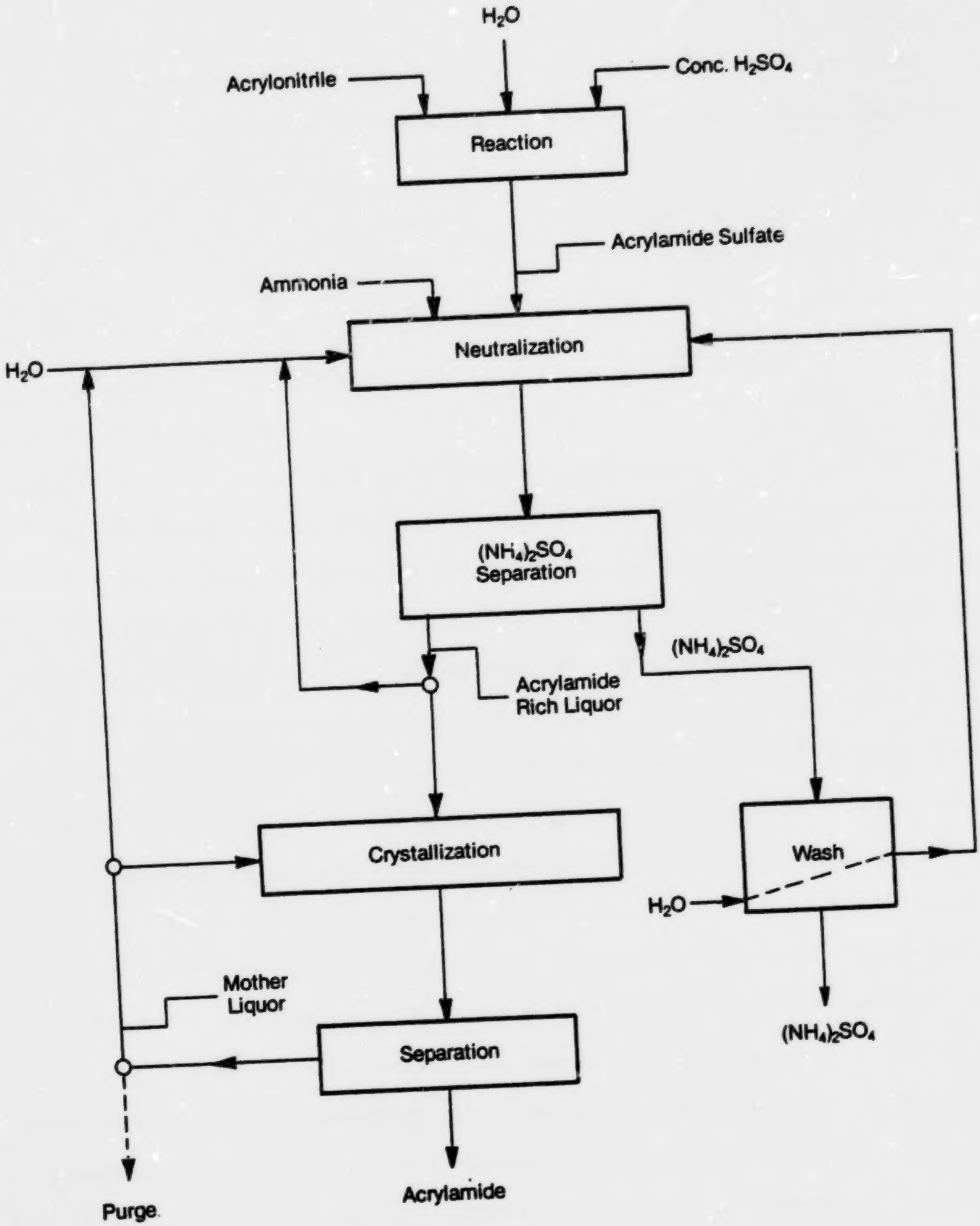
$$CH_2{=}CHCN + HCl \xrightarrow{H_2O} ClCH_2CH_2CONH_2$$

(3-chloropropionamide)

$$ClCH_2CH_2CONH_2 + NaOH \xrightarrow{H_2O} CH_2{=}CHCONH_2 + NaCl + H_2O$$

A typical flow sheet for the commercial production of acrylamide is presented in Figure II-1.

Acrylamide is produced in the United States by Dow Chemical Company in liquid form, by American Cyanamid Company in both solid and liquid forms and by Nalco Chemical Company in liquid form. The total U.S. production of acrylamide in 1978 was reported to be 34,000 metric tons (MacWilliams 1978). Estimated European production for the same period was 16,000 metric tons and Japanese production was 44,000 metric tons.

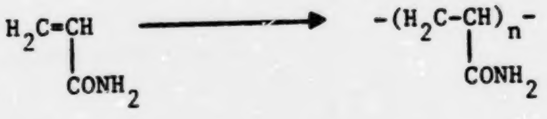


H2O
Acrylonitrile
Conc. H2SO4
Reaction
Acrylamide Sulfate
Ammonia
H2O
Neutralization
(NH4)2SO4 Separation
(NH4)2SO4
Acrylamide Rich Liquor
Crystallization
Wash
H2O
(NH4)2SO4
Mother Liquor
Separation
Purge
Acrylamide

Adapted from Bikales and Kolodny (1963).

Figure II-1 Flowsheet for production of acrylamide.

Acrylamide can readily undergo polymerization and copolymerization reactions, as shown below (Bikales and Kolodny 1963, MacWilliams 1978).



$$H_2C{=}\underset{\displaystyle CONH_2}{CH} \longrightarrow -(H_2C{-}\underset{\displaystyle CONH_2}{CH})_n-$$

acrylamide polyacrylamide

This reaction may be used to produce a broad spectrum of homopolymers and copolymers of controlled molecular weight and performance characteristics, making the chemical very useful. Acrylamide and polyacrylamide are used in the production of flocculants for clarification of potable water and in the treatment of municipal and industrial effluents. Acrylamide and acrylamide polymers find application as additives for enhanced oil recovery, in the production of ores and coal, in the metal industry and in grouting operations. Acrylamide polymers are also used to dissipate fog and stabilize soil (NIOSH 1976), to increase dry strength in paper and paper boards, as components in adhesives and binders, in food packaging, for coating metals and in photography. Smaller quantities are used to introduce hydrophilic centers into oleophilic polymers for promoting adhesion, increasing the softening point and solvent resistance of resins and introducing a center for dye acceptance. Acrylamide is used frequently as a component of photopolymerizable systems. In experimental biomedical research, polyacrylamide gels are widely used for chromatography and electrophoresis.

Analytical methods used for measuring acrylamide include spectrometry, polarography, gas liquid chromatography and electron-capture gas chromatography (MacWilliams et al. 1965, Croll 1971, NIOSH 1976, Poole et al. 1981.

C. Summary

Acrylamide (CH_2=$CHCONH_2$) is a white crystalline solid which is highly soluble in water. The acrylamide molecule has two functional groups: the vinyl group (CH_2=CH) and the amide group ($CONH_2$). Both groups may undergo numerous chemical reactions, but the most important is polymerization. This reaction can be used to produce a broad spectrum of homopolymers and copolymers with a variety of performance characteristics. Such products find wide application in industry and science. Acrylamide is manufactured from acrylonitrile, and in 1978 the total U.S. production of acrylamide was 34,000 metric tons.

III. TOXICOKINETICS

A. Absorption

Miller et al. (1982) administered doses of 1, 10 or 100 mg/kg of ^{14}C-acrylamide (orally, in water) to adult male Fischer-344 rats (three rats per dose), and measured the excretion of label in urine and feces. For each of the three doses, only about 6% of the dose appeared in the feces (4.8% within 24 hours). The remainder was recovered either in the urine (65% to 82%) or in the tissue (total recovery of label was about 90%). Similar results were obtained when 10 mg/kg of acrylamide was given to rats intravenously: 5% to 6% of the label appeared in feces, with the remainder being recovered in urine (71%) or tissues (97% total recovery). The similarity of the excretion patterns following oral and intravenous dosing indicates that acrylamide absorption from the gastrointestinal tract is rapid and complete and does not depend upon dose or concentration.

B. Tissue Distribution

Hashimoto and Aldridge (1970) measured the tissue distribution of radiolabel at various times following intravenous injection of 1-^{14}C-acrylamide (100 mg/kg, 67.2 µCi/mmol) into male Porton strain rats. The results are shown in Table III-1. Label was found distributed throughout the body, and was present in all tissues examined. At all times the highest

Table III-1 Distribution of (^{14}C) in Tissues After a Single Intravenous Dose of (1-^{14}C) Acrylamide (100 mg/kg)

Time after administration	Tissue	(nmol/g tissue)		
		Homogenate	"Free and soluble"	Protein bound
24 hours	Blood	1,280	160	938
	Plasma	63	0	---
	Brain	262	105	156
	Spinal Cord	175	44	110
	Sciatic Nerve	149	60	---
	Liver	368	70	323
	Kidney	508	195	280
4 days	Blood	1,360	7.4	920
	Plasma	26	0	---
	Brain	151	20	95
	Spinal Cord	131	19	89
	Sciatic Nerve	100	16	89
	Liver	142	20	158
	Kidney	248	66	181
14 days	Blood	772	1.8	920
	Plasma	6	0	---
	Brain	72	3.3	49
	Spinal Cord	71	2.8	44
	Sciatic Nerve	57	0	---
	Liver	57	1.6	61
	Kidney	91	1.2	76

Each value is the mean from three experiments. The "free and soluble" is that remaining in solution after treatment with 5% trichloroacetic acid.

Adapted from Hashimoto and Aldridge (1970).

content (up to 1,360 nmol/g tissue) was found in blood, with progressively lower amounts in kidney, liver, brain, spinal cord, sciatic nerve and plasma. In all tissues the majority of label was precipitable with 5% trichloroacetic acid. Assuming that incorporation of $^{14}CO_2$ derived by metabolism of 1-^{14}C-acrylamide (see Section C., below) into macromolecules is negligable, this indicates that acrylamide was covalently bound to proteins or other cellular macromolecules. Subcellular fractionation of brain and liver samples taken at 24 hours showed that all fractions (nuclear, mitochondrial, microsomal, soluble) contained radiolabel, and the specific activity of proteins was similar in each of these fractions.

Ramsey et al. (1984) studied tissue distribution of radiolabel in male Fisher-344 rats that received 13 daily doses (either 0.05 mg/kg or 30 mg/kg) by gavage of 1,3-^{14}C-acrylamide. The form of the label (parent, metabolite, adduct) was not determined. The results are shown in Table III-2. The highest concentration of label was found in red blood cells, with lower amounts found in all other tissues examined. The ratio of the tissue content of label in animals receiving 30 mg/kg/day to that in animals receiving 0.05 mg/kg/day was about 600:1 (the same as the ratio of the doses), indicating that tissue levels are linearly related to dose.

Miller et al. (1982) measured tissue distribution of radiolabel at various times following intravenous injection of 2,3-^{14}C-acrylamide into Fischer-344 rats (three rats/time). Results were expressed as the percentage of the dose found in the total mass of each tissue. The largest percentage of the label was found in muscle (48%) with progressively lower percentages found

Table III-2 Distribution of ^{14}C in Tissues Following 13 Daily Oral Doses of 0.05 mg/kg or 30 mg/kg of 1,3-^{14}C-Acrylamide

Tissue	30 mg/kg	0.05 mg/kg	Ratio
Brain	53.5[a]	0.083	641
Liver	87.7	0.144	610
Kidneys	70.4	0.129	546
Testes	67.1	0.072	943
Epididymis	70.6	0.134	526
Sciatic Nerve	54.0	0.086	631
Carcass	47.6	0.076	628
Skin	39.1	0.071	554
RBC	383.7	1.263	304
Plasma	16.5	0.015	1,089
			Mean = 647 ± 220

[a] Values are expressed as μg equivalents ^{14}C-acrylamide per g tissue, and are the mean values from four rats.

Adapted from Ramsey et al. (1984).

in skin (15%), blood (12%) and liver (7%), and smaller amounts being found in kidney, intestine, lung, brain, spinal cord and sciatic nerve. The specific radioactivity (μmol ^{14}C/g tissue) was the same in neural and non-neural tissue.

Acrylamide also distributes freely across the placenta and enters fetal tissues. Edwards (1976) administered a single intravenous dose of acrylamide (100 mg/kg) to pregnant rats of the Porton strain on day 14 or 21 of gestation. One hour after dosing, the rats were decapitated, the fetuses were removed and homogenized and the concentration of acrylamide (as α-unsaturated carbonyl compounds) was measured in the homogenates. The resulting value was 1.4 μmol/g. The concentration of acrylamide in the blood of male rats that had received the same dose was 1.3 μmol/g. This indicates that the placenta does not serve as a barrier to the distribution of acrylamide.

In a similar study, Ikeda et al. (1983) measured the distribution of ^{14}C-acrylamide in fetuses of Osborne-Mendel rats, New Zealand rabbits, pure-bred beagle dogs and FDA strain Hormel miniature pigs. Acrylamide was administered to the pregnant animals one to two days before parturition. Doses were 10 mg/kg for rats and 5 mg/kg for the other species. After one hour (in rats) or two hours (in other species), the fetuses were removed by Caesarean section, weighed and analyzed for radioactivity. The results revealed essentially uniform distribution of the isotope throughout the litter, suggesting that the placenta did not act as a barrier to distribution of the isotope from maternal circulation into the fetus. There was no significant difference in the total uptake of isotope attributable to the position of the fetus within the uterus in any of the species studied.

Similarly, there was no difference in the uptake of acrylamide by individual organs or tissues (blood, brain, liver, kidney, lung and viscera) in either dogs or pigs which was attributable to the position of the fetus in the uterus.

C. Metabolism

As noted in Section II., acrylamide is electrophilic and undergoes reactions with nucleophiles. Several reports indicated that, in tissues, acrylamide undergoes condensation with the sulfhydryl group of glutathione. Hashimoto and Aldridge (1970) measured the effect of a single oral dose of acrylamide (100 mg/kg) on the non-protein sulfhydryl content of several tissues in rats. The results, shown in Table III-3, revealed a significant decrease in the non-protein sulfhydryl content of brain, spinal cord and liver, four hours after the dose of acrylamide. Levels returned to near normal by 48 to 72 hours after the dose.

Edwards (1975a) administered a single intravenous dose of acrylamide (100 mg/kg) to adult male Porton strain rats, and measured liver glutathione levels as a function of time. Liver glutathione concentration fell rapidly, reaching a minimum of about 36% of control between two and four hours. Levels returned to normal by 10 to 24 hours after the dose.

Miller et al. (1982) administered 2,3-^{14}C-acrylamide orally (100 mg/kg) to rats and analyzed (by thin layer chromatography) the nature of radioactive excretion products appearing in urine. The major product (48% of total

Table III-3 Concentrations of Non-Protein Sulfhydryl in Tissues After a Single Oral Dose of Acrylamide

	Non-protein sulfhydryl (μmol/g tissue)		
	Brain	Spinal Cord	Liver
Control	1.70±0.03	1.22±0.07	6.95±0.33
4 hours	1.26±0.07[a] (74±4.1)	0.83±0.04[a] (68±4.1)	3.19±0.36[a] (46±16)
24 hours	1.32±0.04[a] (78±2.2)	0.92±0.08[b] (75±6.6)	6.56±0.42 (95±11)
48 hours	1.64±0.09 (96±5.3)	1.15±0.08 (94±6.6)	6.45±0.43 (93±14)
72 hours	1.72±0.04 (101±2.2)	1.20±0.12 (98±9.9)	5.70±0.19 (82±6)

Acrylamide was given by oral route (100 mg/kg). Each value is the mean ± S.E. of the results for five rats. The values in parentheses are the percentages of the respective controls.

[a] $P<0.01$.
[b] $P<0.025$.

P values were not supplied by the authors, but were calculated from the data using Student's t-test (two tailed).

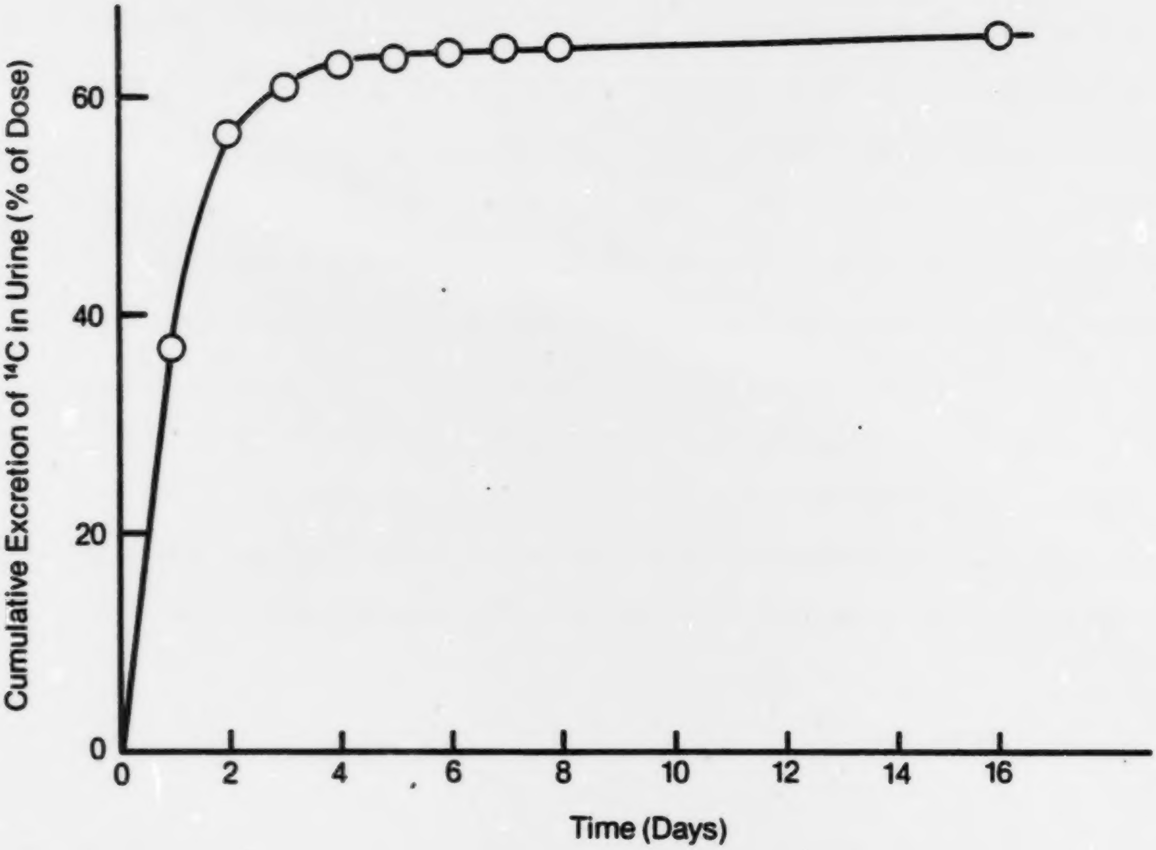
Adapted from Hashimoto and Aldridge 1970.

urinary label) was the mercapturic acid N-acetyl-S-(3-amino-3-oxypropyl) cysteine (NAAOC). Ramsey et al. (1984) found that 70% of urinary ^{14}C was NAAOC following oral or intravenous administration of 1,3-^{14}C-acrylamide. This product is derived by metabolism of the adduct formed by glutathione and acrylamide (Dauterman 1980). Miller et al. (1982) noted several other urinary metabolites (accounting for 2% to 8% of the radioactivity) which did not contain the sulfhydryl group.

Dixit et al. (1982) performed a similar experiment, in which adult male rats (strain not specified) were given single oral doses (by stomach tube) of 25, 50 or 100 mg acrylamide/kg. Urine was collected over 24 hours and metabolites were analyzed by thin layer chromatography. Two thioether compounds were detected in the urine of acrylamide-treated rats. By comparison with synthetic standards, one of the metabolites was identified as a mercapturic acid, and the other resembled cysteine-S-propionamide.

D. Excretion

Acrylamide is excreted via several paths, but the principal route is urinary. Hashimoto and Aldridge (1970) administered 100 mg/kg of ^{14}C-acrylamide to two male Porton strain rats by intravenous injection and measured ^{14}C appearance in urine. The results (Figure III-1) revealed that over 60% of the label was excreted via the urine, mostly within three to four days.



Adapted from Hashimoto and Aldridge (1970).

Figure III-1 Excretion of (^{14}C) in Urine Following a Single Intravenous Dose of 1-^{14}C-Acrylamide (100 mg/kg). Results are the mean of data from two rats.

Miller et al. (1982) administered ^{14}C-labeled acrylamide in water orally to adult male Fischer-344 rats in doses of 1, 10 or 100 mg/kg (three rats per dose). The animals were then housed individually in metabolism cages, and urine and feces were collected daily. Between 53% and 67% of each dose was excreted within 24 hours, reaching 65% to 82% by seven days. More than 90% of the excreted label was in urine, with only 4.8% appearing in feces by 24 hours, and 6% by seven days. The pattern of acrylamide excretion following intravenous acrylamide administration was very similar to that following oral dosing.

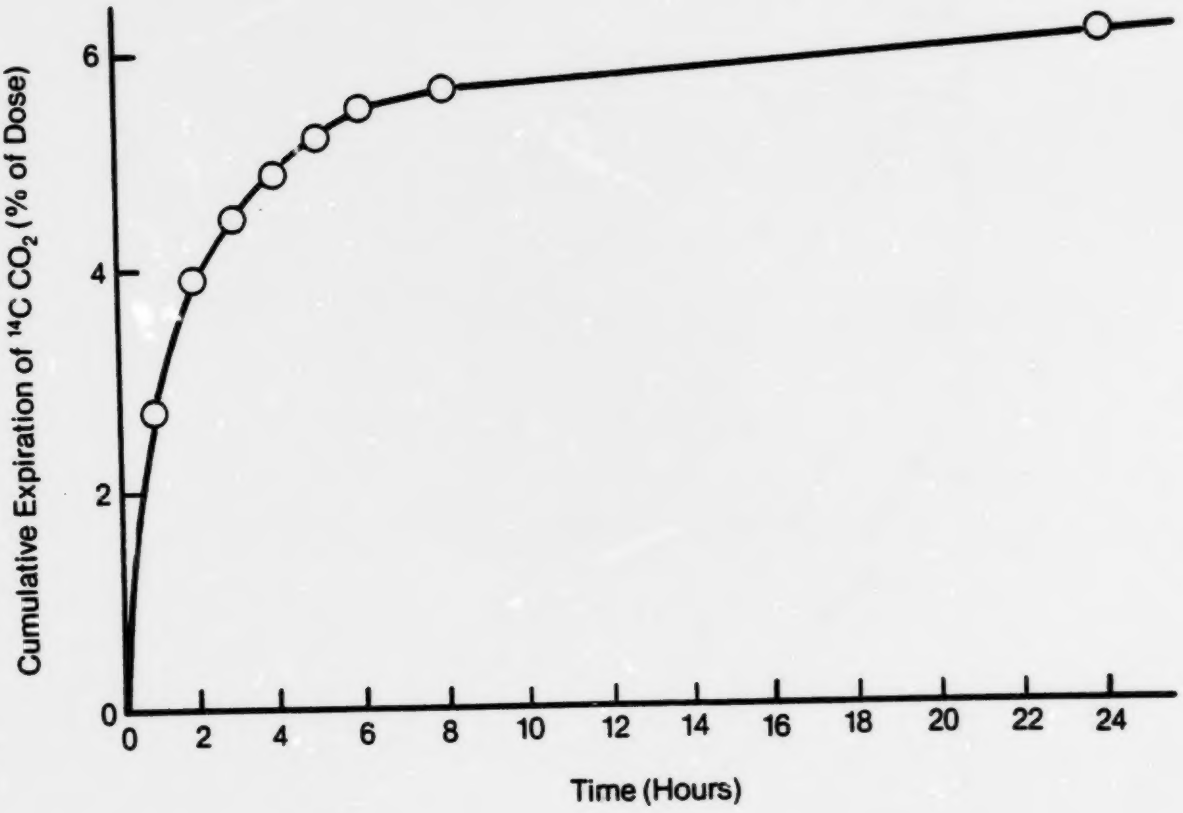
Ramsey et al. (1984) measured urinary excretion of ^{14}C-following oral administration (by gavage) of 1,3-^{14}C-acrylamide to male Fisher-344 rats. One group of four rats received 0.05 mg/kg/day and another group of four rats received 30 mg/kg/day. Over the 13 day dosing period, daily urinary excretion of ^{14}C-label averaged 63%±10% of the daily dose in rats receiving 0.05 mg/kg/day, and 59%±11% in rats receiving 30 mg/kg/day. In two groups of rats (four rats per group) that received a single intravenous injection of 1,3-^{14}C-acrylamide (either 2 mg/kg or 100 mg/kg), urinary excretion of label (over 72 hours) was 67%±2% and 62%±8%, respectively.

In order to study biliary excretion of acrylamide, Miller et al. (1982) anesthetized adult male Fischer-344 rats with urethane and cannulated the common bile duct. A dose of 10 mg/kg of 2,3-^{14}C-acrylamide was administered via the femoral vein, and bile samples were collected every 30 minutes for six hours. By the end of this period, about 15% of the total radioactivity appeared in the bile (1% as unmetabolized acrylamide, the rest as metabolites). Edwards (1975a) also demonstrated biliary excretion of acrylamide following a single intravenous dose (100 mg/kg).

A minor pathway of acrylamide metabolism and excretion involves oxidation of the amide group and expiration as CO_2. Hashimoto and Aldridge (1970) found about 6% of the label from 1-^{14}C-acrylamide administered intravenously to rats appeared in expired air as $^{14}CO_2$, primarily within eight hours (Figure III-2). Ramsey et al. (1984) obtained similar results following intravenous administration of 1,3-^{14}C-acrylamide to rats. Recovery of label in expired air was 4.2±1.9% in rats that received 2 mg/kg and 3.0%±1.1% in rats that received 100 mg/kg. In contrast, Miller et al. (1982) detected no expired $^{14}CO_2$ following intravenous administration of 2,3-^{14}C-acrylamide. This indicates that only the C-1 carbon (the amide group) is oxidized to CO_2.

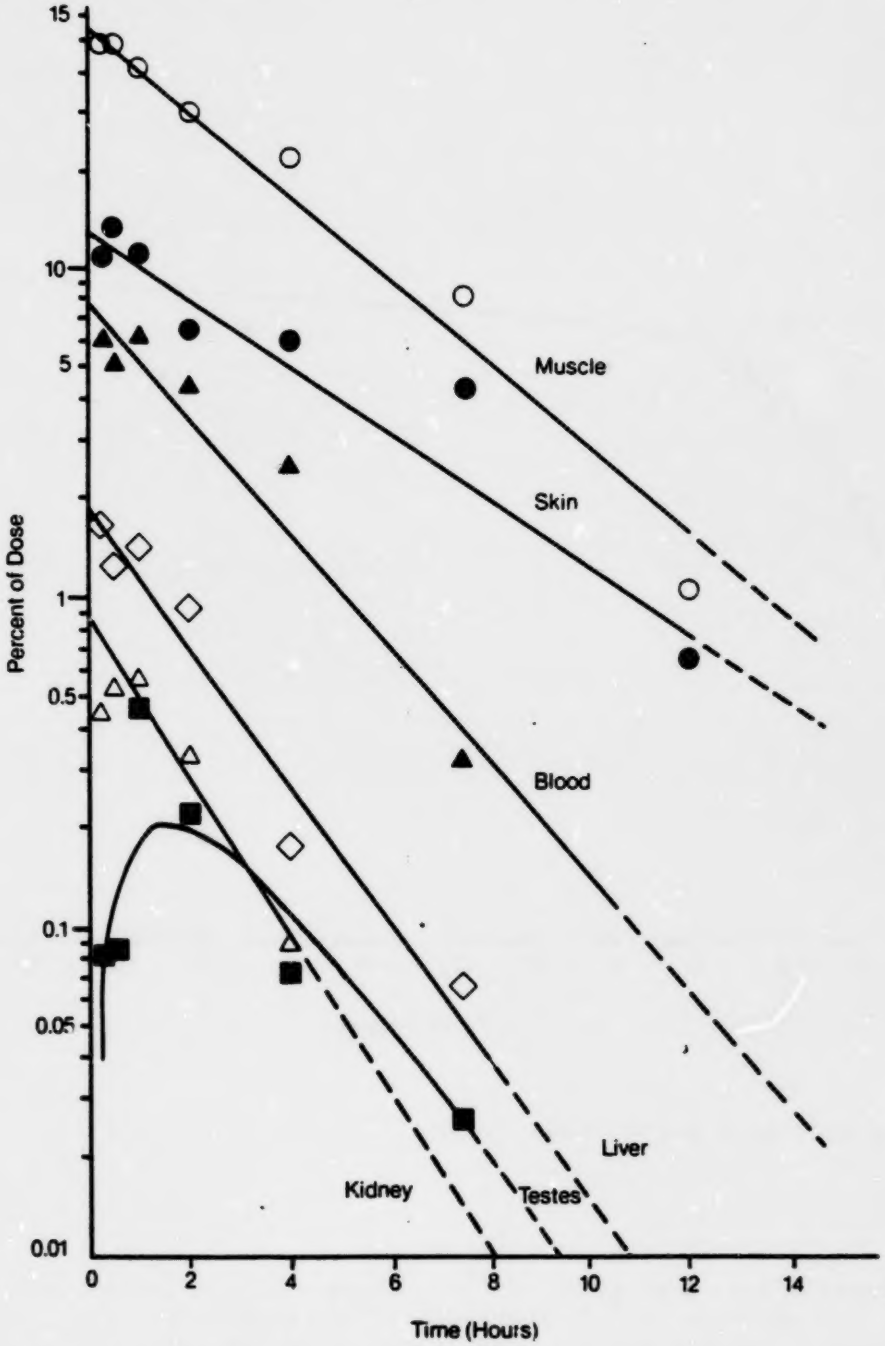
E. Bioaccumulation and Retention

Free (parent) acrylamide is rapidly cleared from the body. Miller et al. (1982) administered, 2,3-^{14}C-acrylamide (10 mg/kg) by intravenous injection to Fischer-344 rats, and measured the amount of parent acrylamide in a number of tissues as a function of time. The results (Figure III-3) indicated that the parent acrylamide content of tissues fell exponentially, with half-times (estimated from the slope of the lines) of one to four hours. No free acrylamide could be detected in any tissue by 24 hours.



Adapted from Hashimoto and Aldridge (1970).

Figure III-2 Expiration of $^{14}CO_2$ After a Single Intravenous Dose of (1-^{14}C) Acrylamide (100 mg/kg). The results are the mean of data from two rats.



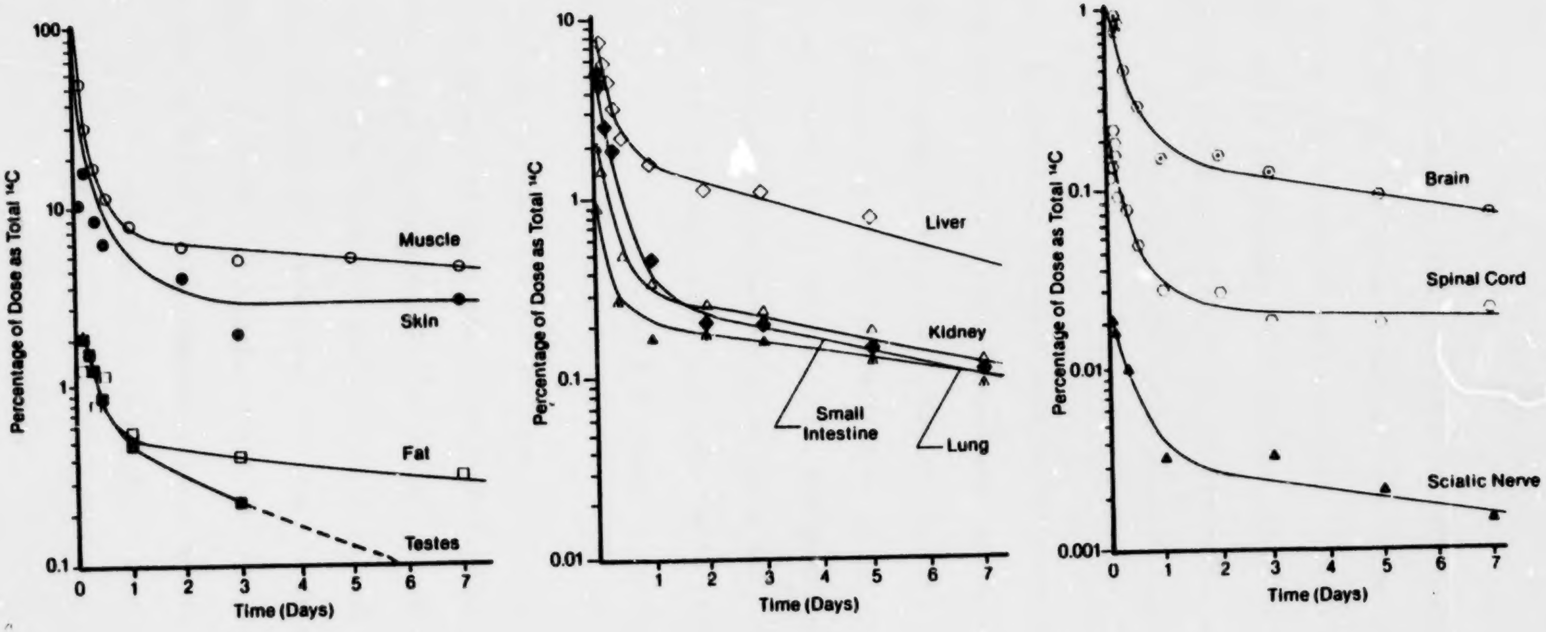
Adapted from Miller et al. (1982).

Figure III-3 Elimination of Parent Acrylamide from Tissues After 10 mg/kg iv. Each symbol represents the mean percentage of total dose as parent obtained from three animals.

Clearance of radiolabel from tissues (an index not only of free acrylamide but of all metabolites and covalent adducts as well) is biphasic and much slower (Figure III-4). The half-time of the first phase in most tissues was less than five hours, and the half-time of the slower second phase ranged up to eight days. Clearance of radiolabel from plasma was similar, but radiolabel was retained in red blood cells (Figure III-5).

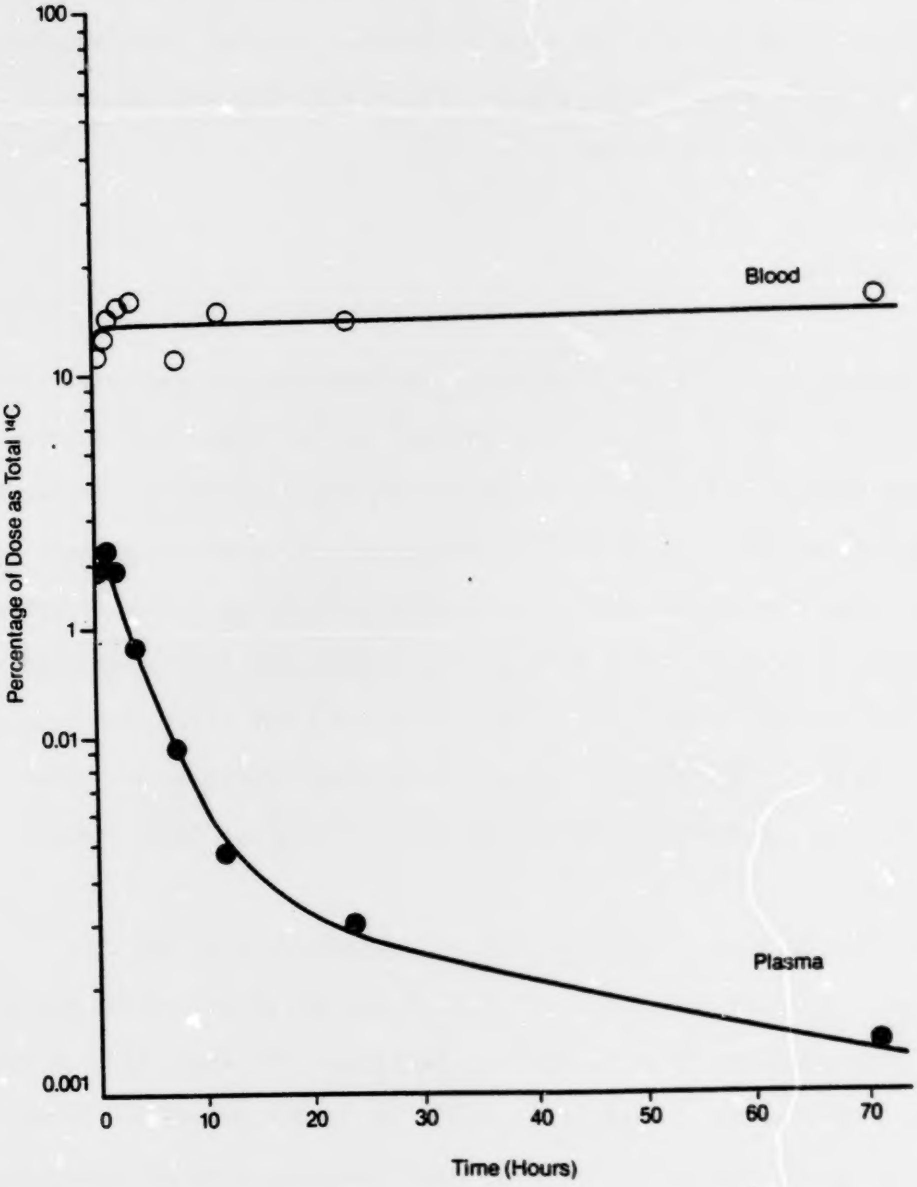
Ramsey et al. (1984) observed similar biphasic clearance of radiolabel from tissues following a single intravenous dose of 1,3-^{14}C-acrylamide (50 mg/kg) to adult male Fischer-344 rats. Analysis of plasma by gas chromatography-mass spectroscopy indicated that the rapid clearance phase represented loss of parent compound, and the slow second phase was due to clearance of metabolites. Red blood cells accumulated label, reaching a maximum value 6 to 12 hours after the dose. This label was not lost from the cells, remaining constant for at least 48 hours.

Hashimoto and Aldridge (1970) measured the distribution of free (non-TCA precipitable) and bound (TCA-precipitable) radiolabel in tissues following a single intravenous dose (100 mg/kg) of 1-^{14}C-acrylamide in rats. The results (Table III-3) indicated that by 24 hours after the dose, most of the radiolabel in tissue was bound, and this label was lost from the tissues rather slowly. After 14 days, the bound radiolabel content of tissues, expressed as percentage of the bound radiolabel after 24 hours, was: blood = 98%, brain = 31%, spinal cord = 40%, liver = 19%, kidney = 27%.



Adapted from Miller et al. (1982).

Figure III-4 Tissue Distribution of Radiolabel Following 10 mg/kg Acrylamide Administered iv. Each symbol represents the mean percentage of total dose obtained from three animals.



Adapted From Miller et al. (1982).

Figure III-5 Blood and Plasma Levels of Radiolabel After 10 mg/kg Acrylamide Administered iv. Each symbol represents the mean percentage of total dose obtained from three animals.

These results demonstrate that a significant portion of an acrylamide dose persists in body tissues for extended periods of time. This may be important in the development of adverse effects following multiple or prolonged exposure to acrylamide.

F. Summary

Acrylamide is rapidly and completely absorbed from the gastrointestinal tract. Only 5% to 6% of an oral dose appeared in the feces, the remainder distributed to tissues throughout the body. The highest content (expressed as nmol/g tissue) was in blood (red blood cells), with progressively lower levels in kidney, liver, brain, spinal cord and nerve, and plasma. When distribution was expressed as percent of the dose in each tissue, the largest amounts were found in muscle (48%), skin (15%), blood (12%) and liver (7%), with less than 1% of the total dose in brain or nerves. Acrylamide freely crosses the placenta in pregnant females and is distributed throughout fetal tissues.

Acrylamide is metabolized primarily by conjugation with cellular glutathione. Cellular non-protein sulfhydryl content in rat brain, spinal cord and liver was significantly ($P<0.01$) decreased four hours after a single oral dose of acrylamide (100 mg/kg). Similarly, liver glutathione levels were decreased to 36% of control two to four hours following a single dose (100 mg/kg) in rats. The major product of acrylamide which appears in urine is the mercapturic acid derived by metabolism of the acrylamide-glutathione adduct.

Excretion of acrylamide occurs via several routes, but the predominant path is the urine. Over 60% of a dose of acrylamide administered to rats, either orally or intravenously, appeared in urine within two to three days. A smaller fraction of the dose appeared in feces (5% to 6%), and about 6% of a dose of acrylamide was oxidized with release of the amide carbon as CO_2.

Free acrylamide is rapidly cleared from the body, being undetectable in rat tissues 24 hours after a single intravenous dose (10 mg/kg). The metabolites and adducts of acrylamide are cleared more slowly, with biphasic kinetics. The half time of the first (rapid) phase of this loss is less than five hours in most tissues, but the half-time of the second (slow) phase is up to eight days. Thus, there is considerable retention of acrylamide in tissues, and this may underlie the cumulative nature of acrylamide toxicity.

IV. HUMAN EXPOSURE

Humans may be exposed to chemicals such as acrylamide from a variety of sources, including drinking water, food, ambient air, occupational settings, and consumer products. This analysis of human exposure to acrylamide is limited drinking water, food, and ambient air because those media are considered to be sources common to all individuals. Even in limiting the analysis to these three sources, it must be recognized that individual exposure will vary widely based on many personal choices and on several factors which there is little control. Where one lives, works, and travels, what one eats, and physiologic characteristics related to age, sex, and health status can all profoundly affect daily exposure and intake. Individuals living in the same neighborhood or even in the same household can experience vastly different exposure patterns.

In the Exposure Estimation section of this chapter, available information is presented on the range of levels of human exposure and intake for acrylamide from drinking water, food, and ambient air for the 70-kg adult male. It is not possible to provide an estimate of the number of individuals experiencing specific combinations of intake from those three sources. However, the Summary section of this chapter provides some insight into the relative contributions of the three sources, especially drinking water, for the range of intake values suggested by the available data.

Exposure Estimation

a. Drinking Water

No information was found in either the Federal surveys or state data on measured levels of acrylamide in drinking water from community water supplies.

Acrylamide is used to produce polyacrylamides, which are used commonly as flocculants in the treatment of drinking water. The current EPA guideline recommends that the residual acrylamide monomer in polyacrylamides used for drinking water treatment not exceed 0.05 percent. This is based on the FDA food additive standards for polymer substances used for food treatment (21 CFR 173.5 and 173.10). The recommended limit of polyacrylamide in drinking water is 1 ppm (Perler, 1984). Therefore, the drinking water limit for residual acrylamide would be 0.5 ppb.

b. Diet

There is insufficient data on acrylamide to estimate dietary intake. Acrylamide is not included in the FDA Market Basket Surveys, nor were any comparable studies found in the literature. Consequently, dietary intake and the contribution of dietary intake to total exposure could not be evaluated.

The Food and Drug Administration (FDA) has issued their Federal Regulation on acrylate - acrylamide resins and modified polyacrylamide resins for use in food treatment (21 CFR 173.5 and 173.10). The polyacrylamide additive or modified polyacrylamide resin should contain not more than 0.05 percent residual acrylamide.

c. Air

No information was obtained on ambient air levels or respiratory intake of acrylamide from EPA's Office of Air Quality Planning and Standards (Pate, 1984) or from the technical literature.

Summary

Sufficient information was not available to assess human exposure to acrylamide or to determine the relative source contribution to total exposure by the three common media (drinking water, food, and ambient air).

REFERENCES

CFR. 1977. Code of Federal Regulations. Title 21, food and drugs, parts 100 to 199. Revised as of April 1, 1977. U.S. Government Printing Office, Washington, DC: 1977.

Pate N. 1984. Personal communication between Dr. Nancy Pate, Pollutant Assessment Branch, Office of Air Quality Planning and Standards, U.S. Environmental Protection Agency and Frank Letkiewicz, JRB Associates, August 1984.

Perler A. 1984. Personal communication between Arthur Perler, Chief-Science and Technology Branch, Office of Drinking Water, U.S. Environmental Protection Agency and Denis Borum, JRB Associates. September 5, 1984.

V. HEALTH EFFECTS IN ANIMALS

In the following section, special attention is given to studies of adverse health effects which occur in animals following oral administration of acrylamide. However, since acrylamide is rapidly and completely absorbed from the gastrointestinal tract (Section III.A.), some studies of adverse effects which follow intraperitoneal or intravenous administration of acrylamide are also included (especially those which provide dose-response data suitable for derivation of quantitative risk assessment factors).

A. General Toxicity

1. Lethality

a. Acute Exposure

McCollister et al. (1964) studied the effect of single oral doses of acrylamide administered to young adult nonfasted animals of several species (female rats, male guinea pigs, male and female rabbits). Acrylamide was administered by intubation in aqueous solution: 2.5% for the rats and guinea pigs, and 12.6% for the rabbits. At a dose of 125 mg/kg, no deaths occurred although some effects were observed (weight loss, lethargy, tremor and pupillary dilation). At a dose of 252 mg/kg, all animals (rats, guinea pigs and rabbits) died within 24 hours. The LD_{50} for each of these species appeared to be in the range of 150 to 180 mg/kg. One rabbit that received 1,000 mg/kg by dermal application of a 12.5% solution of acrylamide, died within two days; two rabbits similarly exposed to 500 mg/kg showed reddening of skin and weight loss. Following instillation into the rabbit eye of a 10% aqueous solution, minor pain and slight conjunctival irritation were produced without any corneal injury. The conjunctiva were completely normal after 24 hours.

A single oral LD_{50} dose for female eight-week-old albino rats of the Porton strain was found to be 203 mg/kg by Fullerton and Barnes (1966). Although a single oral dose of 100 mg/kg produced little more than a fine tremor, repetition of the same dose 24 hours later resulted in death of most rats (number not specified). Other investigations indicated a single oral LD_{50} dose in female rats of 240 mg/kg and in male rats of 277 mg/kg (NIOSH 1976). No information on the strain, age or observation period was given. A single oral LD_{50} dose in male albino mice of 170 mg/kg was reported (NIOSH 1976). The acute oral LD_{50} in five- to six-week-old male mice of DDY-strain was determined to be 106.6 mg/kg by Hashimoto et al. (1981).

Pryor et al. (1983) carried out lethality studies in male Fischer-344 rats weighing 190 to 240 g. Animals were dosed by intubation, and deaths were recorded for the next seven days. At each dose tested eight to ten rats were used. The oral acute LD_{50} was determined to be 202.5 mg/kg.

A summary of the results of these acute studies is presented in Table V-1.

Table V-1 Summary of Lethality Studies of Single Dose Oral Exposure to Acrylamide

Species	Dose (mg/kg)	Effect	Reference
Rat (Male)	203	Acute LD_{50}	Pryor et al. (1983)
Rat (Female)	203	Acute LD_{50}	Fullerton and Barnes (1966)
Rat (Female)	240	Acute LD_{50}	NIOSH (1976)
Rat (Male)	277	Acute LD_{50}	NIOSH (1976)
Rat	252	5/5 Death	McCollister et al. (1964)
Mouse (Female)	170	Acute LD_{50}	NIOSH (1976)
Mouse (5-6 week-old Male)	107	Acute LD_{50}	Hashimoto et al. (1981)
Guinea Pig (Male)	252	4/4 Death	McCollister et al. (1964)
Rabbit (Male and Female)	252	4/4 Death	McCollister et al. (1964)

b. Multiple Exposure

Fullerton and Barnes (1966) added acrylamide to the food of Porton strain rats. Twelve daily doses of 50 mg given over a period of 15 days (average daily dose of 40 mg/kg), produced severe weakness and death. At necropsy, gross distension of the bladder was observed, probably due to retention of urine. Rats (ten-weeks-old) given acrylamide orally at a dose of 25 mg/kg/day, five days/week (average daily dose of 17.9 mg/kg), first developed signs of weakness after the fourth week. A dose of 10 mg/kg, five days/week (average daily dose of 7.1 mg/kg) given for five months, produced no signs of toxicity in six female rats.

Teal and Evans (1982) studied the dose and time dependency of acrylamide lethality in male CD-1 mice. Eleven animals received intraperitoneal injections of acrylamide, 100 mg/kg, five times/week. Within 13 days 100% mortality was observed. A cumulative dose of 600 to 700 mg/kg (six to seven doses over eight to nine days, average daily dose of 75 to 78 mg/kg) produced 50% mortality. Another group of mice received acrylamide at a dose of 60 mg/kg for 22 days (16 doses, cumulative dose 960 mg/kg, average daily dose of 44 mg/kg) followed by a ten-day observation period. Fifty percent mortality was reached after 31 days. There were no deaths in a group of animals receiving 20 mg/kg or in controls. All of the mice that died exhibited tremor and hind-limb weakness. Several mice had distended bladders at necropsy.

Pryor et al. (1983) measured the subacute oral LD_{50} for acrylamide in Fischer-344 male rats (190 to 240 g). Ten rats/group were dosed with acrylamide daily, five days/week for four weeks. The subacute LD_{50} was found to be 32.0 mg/kg.[a] The subchronic LD_{50} also was determined in this study by using fractions of the lowest daily dose that killed 20% of the rats in the four-week subacute study. Rats were dosed five times/week for 15 weeks. The subchronic oral LD_{50} for acrylamide (corrected for part-time dosing regimen[a]) was determined to be 17.0 mg/kg. All rats dosed subchronically with 19.9 mg/kg (average daily dose of 14 mg/kg) acrylamide died. The first deaths occurred during the seventh week of dosing and the last during the fourteenth week of dosing. There were no deaths in the lower dose groups (0, 6.6, 9.6 or 13.8 mg/kg/day, average daily doses of 0, 5, 7 or 10 mg/kg). Acrylamide thus had a subacute oral LD_{50} of about 16% of the single dose oral LD_{50}, suggesting high to moderate cumulative toxicity. The subchronic oral LD_{50} was 53% of the subacute oral LD_{50}, further indicating cumulative toxicity.

A summary of lethality following multiple exposures to acrylamide is presented in Table V-2.

2. General Effects

Gipon et al. (1977) measured the effect of acrylamide on daily food and water intake in inbred female Wistar rats weighing 150 to 170 g. Daily

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[a] H. Tilson, NIEHS, personal communication, 1984.

Table V-2 Summary of Lethality Following Multiple Exposures to Acrylamide

Species	Exposure duration	Average dose (mg/kg/day)	Result	Reference
Mouse	8-9 days	75-78	LD_{50}	Teal and Evans (1982)
Rat	15 days	40	LD_{100}	Fullerton and Barnes (1966)
Mouse	22 days	44	LD_{50}	Teal and Evans (1982)
Rat	4 weeks	32	LD_{50}	Pryor et al. (1983)
Rat	15 weeks	17	LD_{50}	Pryor et al. (1983)
Rat	7-14 weeks	14.2	LD_{100}	Pryor et al. (1983)

intraperitoneal injection of acrylamide (20 mg/kg) for 22 days (cumulative dose of 440 mg/kg) produced a significant ($P<0.05$) decrease in mean body weight (23.1±2.8 g (±SEM) in controls versus 14.6±3.2 g (±SEM) in treated rats). The animals showed a tendency toward decreased food intake, reduced feces production and increased urine production ($P<0.05$), the latter resulting probably from higher water intake. At an acrylamide dose of 50 mg/kg for nine days (cumulative dose 450 mg/kg), a marked reduction in growth ($P<0.05$), paralleled by a lower food intake ($P<0.05$) and decreased feces production was observed in treated animals. Water intake was similar to that of controls and urine production increased significantly ($P<0.05$). In order to determine whether reduced growth was secondary to reduced food intake, growth of animals that received 30 mg/kg/day for 15 days (cumulative dose 450 mg/kg) was compared with growth of pair-fed controls. Despite a parallel reduction in food intake, pair-fed controls maintained a significantly ($P<0.05$) better growth rate than the acrylamide-treated animals.

In similar studies, Schotman et al. (1978) measured daily intake of food and water, and body growth in inbred female albino Wistar rats weighing 150 to 170 g. Saline solution of acrylamide was administered intraperitoneally (50 mg/kg/day) for nine days (cumulative dose 450 mg/kg). The mean body weight in treated animals decreased by 9.2±5.8 g (±SEM) relative to controls ($P<0.05$). The mean daily food intake in treated animals was also reduced, from 81.8±1.6 g/100 g bw[a] in controls to 55.3±3.1 g/100 g bw[a] (±SEM) in treated rats

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[a] The units of food and water intake (g/100 g bw) reported appear incorrect, and probably should be g/kg bw.

($P<0.05$). Daily water intake was reduced from 127.4±2.4 g/100 g bw[a] in controls to 102.9±10.3 g/100 g bw[a] in treated rats. The treated animals were unable to balance on a rotarod at the end of the treatment period and the bladder volumes in these animals were extended five- to six-fold.

Teal and Evans (1982) measured the effect of acrylamide on body weight loss and appetitive behavior in mice. CD-1 mice weighing 30 to 38 g received 15 minutes' access daily to a highly palatable (but nonessential) food, 10% sweetened milk. A single intraperitoneal injection of 100 mg/kg acrylamide produced no effect on body weight and only a slight increase in milk licking. In contrast, food and water consumption decreased by 60% and 70% on day one following injection (saline injected controls averaged 37.5 g body weight, 796 licks for milk licking, 4.6 g daily food intake, and 6.7 mL daily water consumption). The effect of multiple doses was tested in mice that were injected intraperitoneally Monday through Friday for 16 days with either acrylamide (60 mg/kg, average daily dose of 45 mg/kg) ($n=17$) or saline ($n=19$). Saline-treated mice averaged 243 licks and acrylamide-treated mice averaged 582 licks per 15 minutes access period ($P<0.05$), corresponding to consumption of 0.7 and 1.6 mL of milk, respectively. The authors observed that the milk licking test of appetitive behavior preceded other signs of toxicity and suggested the feasibility of using the mouse in behavior toxicology of acrylamide.

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[a] The units of food and water intake (g/100 g bw) reported appear incorrect, and probably should be g/kg bw.

In a similar subchronic study, CD-1 mice received intraperitoneal injections of 20, 60 or 100 mg acrylamide/kg, five times/week for 12 weeks (average daily dose of 14.3, 42.8 or 71.4 mg/kg). In animals receiving 71.4 mg/kg/day there was an accelerated loss of body weight (reaching a value of 65% of that of control groups by day 12). In the 42.8 mg/kg/day dose group, the animals showed a slight decrease in weight but there was a rebound in body weight over the weekends. This allowed the mice to stay within the range of mean body weight of the control group. After acrylamide was discontinued in this group, body weights gradually increased and remained slightly higher than those of control group. The mice receiving 14.3 mg/kg/day did not display a decrease in mean body weight, but maintained and remained 3% to 8% heavier than control.

Huang et al. (1982) investigated whether acrylamide induced abnormal lipid metabolism in rats. Male Sprague-Dawley rats, weighing about 250 g, were divided randomly into two groups of eight rats each. The experimental group received a daily intraperitoneal injection of 50 mg/kg of acrylamide dissolved in saline for nine days. The control group received only saline injections. Food and water were given ad libitum. The body weights of the treated group decreased rapidly, whereas controls registered regular weight gains. The onset of ataxia was noticed after two injections. At the time of sacrifice, urinary retention and enlarged bladders were noticed in the treated animals. The body and tissue weights, as well as cholesterol and phospholipid concentrations in plasma, red blood cell, liver and epididymal fat pad of treated and control rats are presented in Table V-3. In the livers of treated rats, esterified cholesterol decreased, the proportions of linoleate and

Table V-3 Body and Tissue Weights, and Cholesterol and Phospholipid Concentration in Plasma, Red Blood Cell, Liver and Epididymal Fat Pad

	Control (n = 8)	Experimental (n = 8)
Body Weight (bw, in grams)	323.1±15.6	212.5±13.8[a]
Liver weight (% bw)	2.6±0.1	2.9±0.4
Epididymal Fat Pad weight (% bw)	1.0±0.1	0.5±0.3[a]
Cholesterol Concentration		
Plasma (mg/dL)	57.5±5.9	59.3±5.5
Red Blood Cell (mg/mL)	1.1±0.2	1.2±0.2
Liver (mg/g)	1.7±0.3	1.4±0.3[b]
Epididymal Fat Pad (mg/g)	0.7±0.1	1.4±0.4[a]
Phospholipid Concentration		
Plasma (mg/dL)	97.8±12.8	80.7±4.1[b]
Red Blood Cell (mg/mL)	3.2±0.9	3.5±0.6
Liver (mg/g)	25.8±6.5	22.6±9.9
Epididymal Fat Pad (mg/g)	2.0±0.8	6.7±1.7[a]

[a] Significantly different from the control values, P<0.001.
[b] Significantly different from the control values, P<0.05.

Adapted from Huang et al. 1982.

palmitoleate decreased and the proportions of stearate and arachidonate increased. There were only minor changes in the fatty acid profile of the sciatic nerve. A major limitation to this study is that a pair-fed control was not performed. Thus, the altered lipid levels in plasma and tissues might be secondary to decreased food consumption, rather than a direct effect of acrylamide on lipid metabolism.

Sterman et al. (1983) measured the effect of acrylamide on cardiovascular function in rats. Acrylamide was administered intraperitoneally (50 mg/kg) to Sprague-Dawley rats for ten days. Control animals received an equivalent volume of saline. Animals were examined on each day of exposure and on the 12th day (two days after the last dose). In the experimental group, a significant elevation in heart rate (tachycardia) was observed after the first dose of acrylamide, and the heart rate remained significantly elevated on each subsequent testing day. Although there was an elevation in blood pressure (hypertension), it fluctuated and was not statistically significant on all days. The exact mechanism by which acrylamide produced these cardiovascular abnormalities is not known, but autonomic nervous system dysfunction may be a sensitive early marker of acrylamide neurotoxicity.

Pryor et al. (1983) studied the effect of oral dosing of acrylamide on the body weights of male Fischer 344 rats (190 to 240 g at the beginning of dosing). Animals receiving a dose of 13.8 mg/kg/day showed a slight reduction in weight gain which was significant ($P<0.05$) at the end of 15 weeks of dosing. There were no significant effects on thermal sensitivity, motor activity or rectal temperature. Within three weeks after cessation of dosing, the animals recovered their body weight compared to untreated controls.

A summary of the general effects of acrylamide on experimental animals is shown in Table V-4.

B. Target Organ Toxicity

Many adverse health effects resulting from acrylamide exposure are associated with dysfunctions of the central or peripheral nervous systems. The following section summarizes the results of studies which focus on these neural effects. As above, emphasis is placed on studies which involved oral exposure routes, but selected studies which employed parenteral administration are also included. Since the dose of acrylamide required to produce toxic effects is found to depend heavily on the duration of exposure, the descriptions below are organized according to the dosing regimen: Acute (single dose), Short-Term (multiple doses up to about 30 days) and Longer-Term (multiple doses for periods longer than 30 days). Reviews of the neurotoxic effects of acrylamide have been published by Spencer and Schaumburg (1974b) and Tilson (1981).

1. Acute Exposure

Agrawal et al. (1981a) studied the effect of a single oral dose of acrylamide on neurotransmitter levels in the brain. Six-week-old male Fischer

Table V-4 Summary of General Effects of Acrylamide

Species	Average daily dose/duration	Result	Reference
Rats	20 mg/kg for 22 days 50 mg/kg for 9 days	Decreased body wt Decreased body wt Decreased food consumption	Gipon et al. (1977)
Rats	50 mg/kg for 9 days	Decreased body wt Decreased food intake Decreased water intake Impaired motor function	Schotman et al. (1978)
Mice	71.4 mg/kg, one dose	Decreased food/water consumption, increased appetite[a]	Teal and Evans (1982)
	42.8 mg/kg/,16 days	Increased appetite[a]	
Mice	71.4 mg/kg, 12 wks 42.8 mg/kg, 12 wks	Weight loss No significant wt loss	Teal and Evans (1982)
Rats	50 mg/kg, 9 days	Ataxia (2 days) Weight loss	Huang et al. (1982)
Rats	50 mg/kg, 10 days	Tachycardia	Sterman et al. (1983)
Rats	13.8 mg/kg, 15 wks	Decreased wt gain	Pryor et al. (1983)

[a] Appetite assessed by frequency of milk-licking.

rats were administered a single dose of 0, 25, 50 or 100 mg/kg by gavage. The rats were killed 24 hours later and specific brain regions were removed for analysis. Significantly increased binding of ^{3}H-spiroperidol was observed in striatal tissue at all doses of acrylamide ($P<0.05$). In animals that received 100 mg/kg, there was signficantly increased binding of ^{3}H-strychnine in medulla and of ^{3}H-serotonin in frontal cortex ($P<0.05$). No changes were observed in muscarinic receptors in striatum, benzodiazepine receptors in frontal cortex or GABA receptors in cerebellum.

Miller et al. (1983) studied the effect of acrylamide on retrograde axonal transport in male Sprague-Dawley rats. Rats (five animals per dose) were injected intraperitoneally with a single dose of acrylamide (1 to 100 mg/kg) and 15 minutes later the animals were injected with ^{125}I-labeled nerve growth factor (NGF) in a front foot pad. Twelve hours later, ^{125}I radiolabel was determined in ipsilateral and contralateral dorsal root ganglia C_4-C_7. Axonal transport was calculated as the quantity of ^{125}I in ipsilateral tissue minus the amount reaching contralateral tissue through systemic circulation. (The latter was very small.) Acrylamide doses of 1, 5 or 15 mg/kg caused no inhibition of transport, whereas significant inhibition was observed at doses of 25 mg/kg or higher.

In a subsequent report, Miller and Spencer (1984) described the effect of a single dose of acrylamide on retrograde transport in sensory and motor neurons. Adult male CD-1 mice (20 to 25 g) were injected with ^{125}I-labeled tetanus toxin (5 µL, 10 µCi in the gastrocnemius muscle). At 2.5, 5, 7.5 and

10 hours after injection of ^{125}I-labeled toxin, control animals (five/group) were killed, ipsilateral and contralateral dorsal root ganglia (L_3-L_6) and ventral spinal cord (L_3-L_6) were removed, and the ^{125}I-content was measured. Axonal transport was calculated as described above. Experimental animals were injected intraperitoneally with acrylamide (0 to 100 mg/kg) one hour after ^{125}I-toxin injection. Six hours later they were killed and ^{125}I in dorsal root ganglia and ventral spinal cord were measured as before. Acrylamide produced a dose-dependent decrease in ^{125}I-tetanus toxin transport to both dorsal root ganglia and ventral spinal cord. In ventral cord, the threshold dose was less than 25 mg/kg, and a dose of 100 mg/kg completely prevented appearance of label at six hours. In dorsal root ganglia the threshold dose appeared to be about 25 mg/kg, and maximal inhibition (about 50% of control) was achieved at a dose of 50 mg/kg. The effect of acrylamide on the velocity of retrograde transport was assessed by injecting ^{125}I-tetanus toxin at zero, three or six hours after acrylamide injection, and measuring the distance the leading edge of radioactivity traveled in the tibial nerve 1.5 and 2.5 hours later. In control animals, the rate of transport was 6.4 ± 0.9 mm/hour. In animals dosed with 100 mg/kg, the rate of transport averaged 3.9 ± 1.2 mm/hour between 1.5 and 2.5 hours after acrylamide injection, and 1.3 ± 1.0 mm/hour between 4.5 and 5.5 hours after acrylamide injection ($P<0.05$). Doses of 50 and 25 mg/kg also significantly reduced the rate of transport ($P<0.05$) three hours after the dose. The authors concluded a single dose of acrylamide caused profound alterations in retrograde transport which preceded the appearance of structural changes in affected nerve fibers. A NOAEL was not identified, but would appear to be somewhat less than 25 mg/kg.

2. Short-Term Exposure

Prineas (1969) carried out light and electron microscopic studies of nervous system tissue from cats treated with acrylamide. Acrylamide (10 mg/kg) was administered daily by subcutaneous injection to five cats (2.5 to 4 kg). One cat was killed on day 11 of treatment. No neurologic signs (ataxia, hind limb weakness) had developed at this time, but electron microscopic examination of the tibial nerve revealed occasional fibers with increased numbers of neurofilaments and enlarged or swollen mitochondria. In the other cats, neurologic signs developed between days 17 and 22 of treatment. These cats were killed between days 22 and 49. Microscopic examination of intramuscular and tibial nerves from one cat killed on day 22 showed fragmented axons and myelin-ovoid formations. In animals examined after 32 or more days of treatment, sections of upper cervical spinal cord had degenerating myelin sheaths in the spinocerebellar tracts, in the gracile tracts and in the white matter adjacent to the anterior fissure. Electron microscopy revealed increased numbers of neurofilaments in occasional fibers in the tibial nerve, and in a majority of large diameter fibers by day 22 of treatment. Myelin degeneration was usually evident by 49 days. In all animals treated for 32 days, cell bodies in the dorsal root ganglia had some loss of the normal parallel arrangement of granular endoplasmic reticulum, breakdown of polyribosomes, release of ribosomes into the cytoplasm and an increase in the cytoplasm of electron dense material. Similar changes were observed in the anterior horn cells.

Leswing and Ribelin (1969) fed eleven young adult cats (breed and sex unspecified) a diet containing acrylamide in a concentration that delivered a calculated dose of 20 mg/kg/day. After two or three weeks, the authors observed slight weakness of the hind limbs and unsteadiness of the entire posterior half of the body which usually progressed to paralysis of hind limbs. The animals' cries became coarse, indicating to the authors the possible involvement of laryngeal nerves. Conduction velocity in the sciatic nerves decreased by 28% compared to controls. Muscle and nerve spike amplitudes were reduced greatly and higher applied voltages were required to stimulate the nerve. Microscopically, the affected nerves (sciatic-tibial) of all four limbs revealed degeneration of the myelin and axons. Atrophy was evident in nearly all muscles of the caudal limbs, although it was marked only in the muscles of the digits.

In the same study Leswing and Ribelin (1969) fed four monkeys (Cebus albifrons) a diet that delivered 20 mg/kg/day of acrylamide. After about eight weeks of treatment, the dose was raised to 30 mg/kg/day. Bananas injected with a solution of acrylamide in water were used to introduce the chemical into the animals. Measurement of conduction velocity in the sciatic and ulnar nerves revealed decreases of 22% and 20%, respectively.

Bradley and Asbury (1970) investigated the effects of acrylamide in young adult female mice of Balb c Gn/J x SJL/J, and SJL/J strains. Animals were allowed to drink water containing acrylamide at a concentration of 250 ppm ad libitum. Assuming water consumption of 6 mL/day and a body weight of 0.027

kg, the calculated daily intake was 56 mg/kg (Arrington 1972). After 45 days of treatment, the animals were supplied untreated water. None of the animals died during this period. The first onset of neurologic abnormality was observed after 15 days when the animals showed difficulty in grasping and walking; by 25 days, all animals were affected. They dragged their feet while walking, and by 35 days they suffered loss of weight and hair. Within five days after the cessation of acrylamide ingestion, some weight gain was observed. However, some signs of toxicity, as observed by the positioning of the hind limbs, persisted even 20 days post-cessation of acrylamide ingestion. There appeared to be atrophy of hamstring and gastrocnemius muscles. Pairs of animals were killed at intervals between 23 and 165 days after ingestion of acrylamide solution started. Neuropathology revealed that the labeling index in Schwann cells of distal sciatic nerve began to increase by 23 days. (Schwann cell proliferation, as indicated by an increased labeling index, is characteristic of Wallerian-like nerve degeneration or axon loss). Concurrently, a few myelinated fibers were observed to be degenerating (a maximum of 3.4% at 30 days). After 45 days Schwann cell proliferation slowed down. It could not be determined by light microscopy if myelin breakdown preceded proliferative activity of Schwann cell nuclei.

Peripheral neurotoxicity of acrylamide in dogs was assessed by Thomann et al. (1974). Six- to twelve-month-old beagles were given acrylamide orally in gelatin capsules at daily doses of 5 or 15 mg/kg. Three male and three female dogs received 5 mg/kg/day (seven days/week) for 60 days. After three weeks of treatment, they became inactive, displaying sedation, ataxia and muscular weakness which was particularly noticeable in the jaw muscles. After 60 days of treatment, three of six dogs developed occasional swelling and fragmentation of myelin sheaths and axons, especially sciatic nerve distal branches. Additional effects observed in five male and five female dogs treated with 15 mg/kg/day acrylamide for 22 days included dilated pupils, conjunctivitis, salivation, difficulty in breathing, muscular stiffness of hind legs and convulsions. One female dog in the 15 mg/kg/day group died two days after the end of acrylamide treatment. Light microscopic examination of the peripheral nerves (sciatic, fibular, tibial) from one of the surviving animals in this group revealed swelling of the myelin sheaths and fragmentation of axons. The distal branches of the sciatic nerve appeared to be more severely involved than the proximal parts.

Edwards and Parker (1977) described a simple, sensitive and objective method for early assessment of acrylamide neuropathy in rats. Male Porton rats (200 g) were given intraperitoneal injections of acrylamide (50 mg/kg) or saline, three times a week for two weeks (average dose of 21.4 mg/kg/day). Neuropathy was assessed by measuring the spread of hind-limbs when the animals were dropped. After three doses of acrylamide, the landing foot-spread of treated animals was significantly greater ($P<0.02$) than that of controls; after four doses the increases were more significant ($P<0.005$). No significant change in body weight was observed. No obvious change in behavior of animals was seen at four days of treatment, although at seven days some differences were apparent.

Similar results were observed when rats were given acrylamide in the diet. After 14 days on a diet containing 200 ppm (about 20 mg/kg/day) or after seven days on a diet containing 400 ppm of acrylamide (about 40 mg/kg/day), a significant increase in landing foot-spread was observed. Abnormalities could not be detected by simple observation until after 18 days in the 200 ppm group or 10 days in the 400 ppm group. The landing foot-spread method was quantitative, more sensitive than subjective judgments of behavior, and the sensitivity was comparable to the rotarod test described by Kaplan and Murphy (1972). Unlike the latter test, the landing foot-spread does not need training of rats or special apparatus.

Lowndes et al. (1978) investigated Achilles tendon reflexes and the dynamic discharge from primary muscle spindles in adult cats with acrylamide-induced neuropathy. Cats were given daily injections of acrylamide dissolved in saline at a dose of 30 mg/kg. Five or ten injections (cumulative dose of 150 mg/kg or 300 mg/kg) were given intramuscularly into gluteal or shoulder muscles, the injection site being varied daily. Untreated cats served as controls. Achilles tendon response was assessed by sharp raps with a reflex hammer. Physiologic studies were made under α-chloralose anesthesia. Cats that received the 300 mg/kg cumulative dose of acrylamide developed more severe gait disorders than those given the lower dose (150 mg/kg cumulative). Tendon responses were readily elicited in three of five animals given a cumulative dose 150 mg/kg of acrylamide although the responses were somewhat depressed. In three of five cats that received a cumulative dose of 300 mg/kg, no tendon responses were elicited, and in the remaining two cats the responses were difficult to elicit. In five cats that received a cumulative dose of 150 mg/kg, average dynamic indexes of 13 primary endings of stretched soleus muscle spindles were 20% lower than in normal cats, although this difference was not statistically significant. For 17 primary endings obtained from four cats that received a cumulative acrylamide dose of 300 mg/kg, the average dynamic indexes were about half of that of normal ($P<0.01$). Additionally, the spindles responded to stretch with only 20% to 30% of the normal number of afferent impulses. The authors concluded that the decrease in tendon response in acrylamide neuropathy resulted in part from inadequate activation of motor neurons by spindle afferent discharge.

Gorzinski et al. (1979) studied the effect of acrylamide on a number of parameters (both neural and non-neural) in male and female CDF Fischer 344 rats (254 to 288 g). Acrylamide monomer was administered in drinking water at levels of 0, 1, 3, 10 or 30 mg/kg day for 21 consecutive days. Five males and five females constituted each dose group. The animals were observed daily for overt signs of toxicity. Food and water consumption and body weight were recorded weekly. The onset of neuropathy was tested by the landing food-spread method of Edwards and Parker (1977). At termination, clinical chemistry determinations of serum urea nitrogen (BUN), alkaline phosphatase (AP) and glutamic pyruvic transaminase (GPT) activities were conducted for all animals. All animals were subjected to gross necropsy. Organ weights, body weights and organ-to-body-weight ratios were determined. Histological examinations were done on brain, spinal cord and peripheral nerve (brachial plexus, femoral and sciatic nerves) from all controls, high dose animals and selected animals from the other dose groups. Sections of peripheral nerves from three animals/sex/dose were fixed for electron microscopy, but only tissues from the control and highest dose groups were processed for examination.

Some of the animals receiving 30 mg/kg/day began to exhibit an increase in hind limb splaying by the end of the second week of treatment. Midway through the third week, there was a definite trend apparent in this group. A decreased water consumption and a slight decrease in body weight gain were observed for both sexes. Food consumption decreased significantly in females during the third week. Males receiving 30 mg/kg/day had increased GPT and decreased AP activities at termination. Also, some differences in organ-to-body-weight ratios were observed in the animals receiving 30 mg/kg/day. Treatment-related effects were also noted in the peripheral nerves, but not brain and spinal cord, both at the light and electron microscopic levels. One male rat treated at 10 mg/kg/day also exhibited some abnormalities in peripheral nerve at the light microscopic level of observation. No statistically significant effects were observed in animals receiving lower doses of acrylamide (1, 3 or 10 mg/kg/day). The investigators determined that dose levels of 1 and 3 mg/kg/day for 21 days could be interpreted as NOAELs in both sexes.

Merigan et al. (1982) studied the effect of acrylamide on the visual system in monkeys. Four adult female macaque monkeys (Macaca nemestrina) were used. Three of the monkeys were given acrylamide orally in fruit juice (10 mg/kg/day) five days/week (average daily dose of 7.1 mg/kg). Dosing continued until the monkeys were markedly ataxic (after 33, 34 and 47 doses). The fourth monkey served as the time-matched control and was given the vehicle alone for a period equal to the longest period of administration of acrylamide. One monkey was killed at the end of the dosing period for histopathologic study, and the other two monkeys were studied for more than 90 days after dosing and then killed. Both behavioral and electrophysiological tests were used to assess visual capabilities. In all three monkeys, subchronic exposure to acrylamide caused deterioration in visual capabilities well before the monkeys showed overt signs of toxicity. For example, indices of visual impairment were observed after 20 doses (total dose of 200 mg/kg), well before the onset of motor impairment which began two weeks later after a cumulative dose of 300 mg/kg.

Cessation of dosing resulted in rapid recovery of flicker-fusion thresholds, gradual recovery in evoked potential latency and partial recovery of visual acuity. No pupillary or eye movement abnormalities, and no changes in ocular media or ophthalmoscopic appearance of the retina were found, indicating that the changes observed in visual capabilities reflect pathology in the visual pathways of these monkeys. Since these central changes had not reversed completely within a 90-day post-exposure period, the investigators suggested that these changes may be irreversible.

Sterman (1982) studied the effects of acrylamide exposure on neuronal cell body morphology. Two groups of female Sprague-Dawley rats (49 to 56 days old, 180 to 200 g) were given intraperitoneal injections of acrylamide at doses of 0 or 50 mg/kg/day. Animals were sacrified after six days and lumbar dorsal root ganglia and distal sural and tibial nerves were prepared for morphological examination. Large and small neuronal cell bodies showed a spectrum of remodeling changes that included nuclear eccentricity, cytoplasmic reorganization with an outer mantle of Nissl and an inner perinuclear zone of pigmented bodies and increased perineuronal cells. Distal axons showed little degeneration at this time. The author concluded that the cell body plays an important role in the pathogenesis of dying-back neurotoxic disease, and that there may be direct toxic effect of acrylamide on the perikaryon itself.

Miller et al. (1983) employed the techniques previously mentioned (Section V.B.1.) to study the effect of repeated administration of 15 mg/kg acrylamide on retrograde transport of NGF. Hind limb foot-splay was monitored as a measure of peripheral nerve function. Five doses of 15 mg/kg/day (cumulative dose of 75 mg/kg) markedly inhibited NGF transport, and the maximal inhibitory effect occurred after 15 daily doses (cumulative dose of 225 mg/kg). Hind limb foot-splay was not affected until 15 daily doses had been administered (cumulative acrylamide dose of 225 mg/kg) and then increased with subsequent doses of acrylamide in a dose-related manner. This study showed that axonal transport is a more sensitive index of acrylamide exposure than is the foot-splay test, but whether alterations in retrograde transport *per se* represent neurotoxicity remains to be established.

Jakobsen and Sidenius (1983) investigated the effects of acrylamide on axonal transport in rats using a procedure somewhat different from Miller et al. (1983). A group of 46 male Wistar rats (15 weeks old, 245 to 390 g) were dosed intraperitoneally with acrylamide (50 mg/kg) twice a week (average daily dose = 14.3 mg/kg/day) until cumulative exposure reached 50, 100, 200, 300 or 500 mg/kg. The left and right fifth lumbar spinal root ganglia were exposed and injected with ^{35}S-methionine in order to label newly synthesized proteins. After nine hours, the left and right sciatic nerves were exposed and two ligatures, 9 mm apart, were made. After 15 and 24 hours, respectively, the right and left sciatic nerves were removed, frozen and divided into five 3 mm segments (one proximal, one distal, three interligatures). The amount of trichloroacetic acid/phosphotungstic acid insoluble label was measured in each segment, and the accumulation of label in the proximal and distal segments was calculated relative to the interligature radioactivity.

Accumulation of label in the distal segment, measured after 15 hours, was not significantly affected except at the highest dose. In contrast, accumulation of label in the distal segment measured at 24 hours was reduced in a dose-dependent fashion at all points except the lowest dose (50 mg/kg total). These observations were interpreted to mean that the amount of labeled material in retrograde transport was reduced (due to retention in the distal axon), but the velocity of retrograde transport and the turnaround time remained normal. These effects preceded but were correlated with the appearance of neurological symptoms in the animals.

Similar measurements on the proximal segment revealed no evidence of an effect on anterograde transport, except at the highest dose (500 mg/kg total). This result was confirmed and extended by Sidenius and Jakobsen (1983). A group of 38 male Wistar rats (15 weeks old, 285 g to 400 g) were dosed with acrylamide intraperitoneally as above. After cumulative doses of 100 to 60 mg/kg, either ^{3}H-proline or ^{35}S-methionine was injected into the dorsal ganglion and the distribution of label in the sciatic nerve was measured after 2 hours, 4 hours, 10 days or 26 days. From the area, shape and position of the peak of labeled material at these time intervals, it was concluded that neither the velocity nor the amount of material carried by the fast-component (FC) or slow-component-b (FCb) of anterograde transport was affected. For slow-component-a (SCa), the velocity of transport was not affected, but the amount of material was reduced by up to 53% at the highest dose (600 mg/kg total). The authors concluded that abnormalities in SCa could play a role in acrylamide-induced neuropathy.

A summary of the short-term studies of acrylamide effects is presented in Table V-5.

3. Longer-Term Exposure

The effect of administration of acrylamide by intravenous, intraperitoneal, intramuscular, oral or subcutaneous routes was studied in cats by Kuperman (1958). Daily doses of 1, 2, 5, 10, 15, 25, 40 or 50 mg/kg were administered. Groups of 3 to 11 cats (strain, sex and age unspecified) were used at each

Table V-5 Summary of Acrylamide Effects
Following Short-Term Exposure

Animal species	Average daily dose (mg/kg)	Length of exposure	Effects observed	Reference
Cat	10	11 days	No external neuropathology, some change in nerve by electron microscopy	Prineas (1969)
		17-22 days	Signs of neuropathy	
		22-49 days	Neuropathology evident by light or electron microscopy	
Cat	20	2-3 weeks	Hind limb weakness, decreased nerve conductor velocity, neuropathology by microscopy, atrophy	Leswing and Ribelin (1969)
Mouse	56	20-25 days	Gait disorder	Bradley and Asbury (1970)
		25 days	Neuropathology	
		45 days	Muscle atrophy	
Dog	5	21 days	Ataxia, weakness	Thomann et al. (1974)
		60 days	Demyelination	
	15	22 days	Ataxia, weakness, stiffness, convulsions	
Rat	21.4	3-14 days	Increased hind footspread when dropped	Edwards and Parker (1977)
Cat	30	5 days	Depressed tendon reflexes	Lowndes et al. (1978)
		10 days	Gait disorder, absent tendon reflexes, reduced dynamic index	

continued-

Table V-5 - continued

Animal species	Average daily dose (mg/kg)	Length of exposure	Effects observed	Reference
Rat	1-3 10 30	21 days 21 days 14-21 days	NOAEL Neuropathology (1/10) Hind limb splaying, weight loss, neuropathology by light and electron microscopy	Gorzinski et al. (1979)
Monkey	7.1	26 days 35-46 days	Visual impairment Ataxia, motor impairment	Merigan et al. (1982)
Rat	50	6 days	Altered neuronal cell body morphology	Sternman (1982)
Rat	14.3	1-5 weeks	Decreased retrograde axonal transport	Jakobsen and Sidenius (1983)
Rat	14.3	1-6 weeks	Altered anterograde transport in component SCa only	Sidenius and Jakobsen (1983)
Rat	15	5-15 days	Reduced axonal transport, no foot-splay until 10 days	Miller et al. (1983)

dose. Effects of chronic poisoning, as measured by the development of ataxia, appeared at roughly identical total doses and after equivalent latent periods, irrespective of the route of administration. The length of the latent period from start of the treatment was inversely related to the dose and varied between 125 days (with the 1 mg/kg/day dose) and 2 days (with the 50 mg/kg/day dose). Clinical signs appeared when the animals had received an average of 102 mg/kg total dose over a period of a few days to four months. The continued daily administration of acrylamide after the appearance of postural, motor and behavioral disturbances resulted in development of profound weakness and culminated in death. The average cumulative dose required for the moribund stage of poisoning was 320 mg/kg (about three times the cumulative dose required to produce ataxia). Necropsy of cats did not reveal any characteristic morphological or microscopic pathology of brain or spinal cord. Microscopic examination of other organs, urinalysis, blood chemistry and liver function tests were also negative. Administration of a single dose of 65 mg/kg, or repeated administration of 15 to 40 mg/kg produced characteristic electroencephalographic (EEG) changes, including a pronounced increase in wave frequency, complete abolition of large voltage slow waves and desynchronization from all areas of the cerebral cortex.

The effect of feeding acrylamide mixed with polyacrylamide polymer (polymer by itself is essentially non-toxic) in the diet was studied in adult Dow-Wistar rats (McCollister et al. 1964). Groups of ten male and ten female eight-week-old rats were maintained on diets containing 0, 0.3, 0.9, 3, 7, 9, 11, 30 or 40 mg/kg acrylamide monomer. At the end of the experiments, the animals were killed and necropsied. After 90 days, animals receiving 0.3, 0.9 or 3 mg/kg/day exhibited no abnormality in gross appearance, behavior, food consumption, growth nor gross and microscopic appearance of tissues. Similarly, rats maintained on 7.0 or 11.0 mg/kg/day acrylamide for 189 days did not exhibit any toxic effects. In the groups maintained on 30.0 mg/kg/day in the diet, one animal demonstrated loss of control of hind quarters after 21 days, and five others were affected with the same condition within 90 days. By 42 days, all of the males in the 30 mg/kg/day groups were dead with only four of ten females surviving. These were taken off the acrylamide diet and completely recovered by 57 days on the control diet. In the animals receiving 40 mg/kg/day acrylamide, loss of hind quarter function was observed after 14 days of dosing, with complete loss of control in most rats by 24 days. On the 25th day, the animals in this group were put on a control diet. Recovery was gradual, but complete by 55 days on the control diet.

A one-year feeding study of cats (strain unspecified) receiving various concentrations of acrylamide in the diet was reported by McCollister et al. (1964). The diet, fed ad libitum, delivered calculated daily doses of 0.03, 0.1, 0.3, 1.0, 3.0 or 10.0 mg/kg. Cats were fed acrylamide five days/week (average daily doses of 0.02, 0.07, 0.2, 0.7, 2.1 or 7.1 mg/kg) and observed for weight, gross appearance and behavior. Two animals per dose and three controls were used. Two of the three controls died within the first month, however. The third was terminated after 171 days. For comparison purposes another cat was added as a control at terminal autopsy. At a daily dose of 7.1 mg/kg, the cats developed weakness in their hind quarters by 26 days (total dose 200 mg/kg); by 40 days, loss of control of hind legs was observed; and by 52 days (total dose of 370 mg/kg), loss of control of all four legs was observed. At this time one of the cats was killed and necropsied; the other was put on an acrylamide-free diet. The latter recovered completely within 53 days. Cats fed on the 2.1 mg/kg/day dose developed hindquarter trembling at 26 days and hind quarter weakness by 68 days. This weakness persisted during a dosing period of 299 additional days. One of the two cats given a dose of 0.7 mg/kg developed signs of weakness in the hind legs by 240 days of dosing. One of the animals dosed at 0.2 mg/kg/day died at 106 days, apparently for reasons unrelated to the acrylamide treatment. The other cat exhibited no adverse reaction throughout the year. The four animals at the two lowest doses (0.02 and 0.07 mg/kg/day) died of spontaneous diseases before 102 days. Light microscopic examination of the brain, spinal cord and sciatic nerve revealed no evidence of adverse effects of acrylamide ingestion at any of the doses, including the 10 mg/kg/day dose. A NOAEL of 0.2 mg/kg average daily dose is indicated by the results of this study, although the small number of animals (two per dose) and the high death rates limit the usefulness of this study.

McCollister et al. (1964) also studied the effects of acrylamide on female monkeys (strain and age unspecified) exposed for one year (363 days). One monkey was used per dose of 0.03, 0.1, 1, 3, or 10 mg/kg/day; two monkeys were given 0.3 mg/kg/day and two monkeys served as controls. The 10 and 3 mg/kg doses were given intragastrically, and lower doses were administered in bananas. Animals received acrylamide 5 days/week (average daily doses of

0.02, 0.07, 0.2, 0.7, 2.1 or 7.1 mg/kg). Monkeys maintained on 0.02, 0.07, 0.2 or 0.7 mg/kg acrylamide/day exhibited no evidence of adverse effects as judged by growth, appearance, behavior, periodic neurologic examination, terminal hematologic (no details given) examination, or gross and microscopic study (at necropsy) of liver, kidney, brain and spinal cord tissues. The monkey that received 2.1 mg acrylamide/kg/day sometimes exhibited knee jerk and pupillary reflexes that were more sluggish than in controls, but this was considered to be insignificant by the authors. The monkey maintained on the acrylamide dose of 7.1 mg/kg/day exhibited some weakness of the hind quarters at 29 doses (cumulative dose of 290 mg/kg), and extreme weakness after 49 doses (cumulative dose of 490 mg/kg). This animal was returned to an acrylamide-free diet on day 70, and complete recovery was observed 54 days later. These results indicated to the authors that doses up to 0.7 mg/kg/day produced no toxic effects, but doses of 2.1 or 7.1 mg/kg/day produced definite signs of neurotoxicity. The NOAEL of acrylamide in the monkey, as suggested in this study, was about 0.7 mg/kg average daily dose.

Fullerton and Barnes (1966) subjected six- to eight-week-old Porton strain male rats to a daily intake of acrylamide in the diet for up to 48 weeks. The diet contained 0, 100, 200, 300 or 400 ppm acrylamide, which resulted in daily intakes of 6 to 9 mg/kg (100 ppm), 10 to 14 mg/kg (200 ppm), 15 to 18 mg/kg (300 ppm) or 20 to 30 mg/kg (400 ppm). Electrophysiological and histological studies were carried out. Conduction velocity in the nerve fibers supplying the small muscles on the plantar surface of the hind paws in ten control rats was 56 meters/second (±5.8 SD) compared with 44 meters/second (±2.2 SD) in 11/15 rats treated at 200 ppm (10 to 14 mg/kg) for six months, or 400 ppm (20 to 30 mg/kg) for two to three months. Animals treated at 400 ppm (20 to 30 mg/kg) had neurologic abnormalities of the hind limbs at about three weeks, that became severe at eight weeks. At 300 ppm (15 to 18 mg/kg), rats developed slight abnormalities at four weeks, which became severe at 10 weeks. At the 200 ppm dose (10 to 14 mg/kg), slight leg weakness appeared by 12 weeks, and by 16 weeks the abnormality had become severe. An additional 30 weeks on this diet did not lead to greater effects. At 100 ppm (6 to 9 mg/kg) the rats began to develop slight leg weakness after 40 weeks on the diet; this weakness did not intensify over the remaining eight weeks of the experiment. Histopathologic findings included extensive degeneration of peripheral nerves, with distal parts more affected than proximal segments. Macroscopic findings at necropsy included distended urinary bladders and wasting of hind limb musculature. Nerve conduction velocity in motor fibers to the muscles of the hind paw was reduced significantly in severely affected animals. When measured again following recovery from severe paralysis, mean conduction velocity had returned to normal level.

Hopkins (1970) investigated the effect of acrylamide in five female and two male baboons (Papio hamadryas) weighing 9.4 to 15.4 kg. Acrylamide was administered in oranges or bananas as a 10% aqueous solution. One of the baboons may not have eaten all the fruit into which acrylamide was injected. This was reflected in the delay in onset of signs of toxicity when compared with the other animals receiving the same dose. Of the remaining six baboons, one was treated with 20 mg/kg/day for 29 days, another with 15 mg/kg/day for

94 days and four others with 10 mg/kg/day over a period of 89 to 192 days. Lack of coordination and weakness of the hind quarters was first observed on the 16th day in the animal receiving 20 mg/kg/day, on the 42nd day in the animal receiving 15 mg/kg/day, and between 42 and 97 days in the animals receiving 10 mg/kg/day. Recovery began 2 to 12 days after cessation of treatment. Wallerian-type degeneration of the peripheral nerves from baboons given 10 mg/kg/day for 118 days and biopsied 19 days later was evident by light microscopic study. Microscopic examination of both motor and sensory nerves revealed marked reduction in the number of myelinated fibers.

In a subsequent report Hopkins and Gilliatt (1971) described electrophysiologic studies performed on the baboons described above. The baboon receiving 15 mg/kg/day acrylamide for 94 days had severely reduced amplitudes in action potentials from the abductor pellicis brevis and extensor digitorum brevis muscles. The magnitude of reduction in action potential amplitude in the median and anterior tibial nerves was unchanged through the first year after cessation of exposure but returned to 80% normal by the end of the second year. The amplitude of the muscle action potential in a baboon given 10 mg/kg/day acrylamide for 115 days was less severely affected and began to return to normal within two to three months after exposure ended. The largest and longest fibers of both motor and sensory nerves degenerated first.

Schaumburg et al. (1974) studied ultrastructural changes in the nervous system of two cats (strain, sex, and age unspecified) given acrylamide in drinking water, ad libitum, at a dose of 3 mg/kg/day. One cat developed a gait disorder after 70 days. The other cat developed a gait disorder after

163 days. Hind foot drop and distal muscle weakness was observed in these cats within seven months. Hind foot toe pad biopsies taken from the animals on the day after the last day of dosing (the 253rd and 295th days, respectively) revealed loss of all types of myelinated fibers in the distal nerves, both sensory and motor. Only a few myelinated nerve fibers were observed in plantar nerve twigs. Most of the fibers were completely degenerated, replaced by bands of Büngner (Schwann cell processes, sometimes associated with regenerative axon sprouts and surrounded by residual basement laminae). Most of the muscle fibers were vacuolated and shrunken, with irregular border changes considered secondary to degeneration.

The authors determined that there was a temporal vulnerability in acrylamide intoxication such that the earliest changes were seen in the pacinian corpsucle axons (sensory), followed by degeneration of muscle spindle primary afferents and extrafusal motor nerve terminals. In addition, this study confirmed earlier findings that acrylamide exposure induces a distal to proximal pattern of degeneration, although it was not possible to determine if the initial damage occurred at the most distal point of the axons.

Burek et al. (1980) evaluated the subchronic toxicity of acrylamide in CDF rats. Acrylamide was administered in drinking water daily for 90 days to ten female rats and 23 to 29 male rats at each dose level (0, 0.05, 0.2, 1, 5, or 20 mg/kg/day). Ten males per group were held for 144 days after the treatment period for recovery studies. Additional rats were included for interim kills and ultrastructural evaluations during the study.

The onset of neuropathy was evaluated by the limb-splaying method. Hematological parameters measured at day 76 of exposure (in seven of ten animals from both the control and 20 mg/kg group) and at the termination of exposure (all groups) included: packed cell volume (PCV), total red cell count (RBC), total and differential white cell count (WBC) and hemoglobin concentration (Hgb). Urinalyses were performed on day 76 in controls only. Attempts to do so in the 20 mg/kg animals were unsuccessful. Urinalyses were performed in all groups at the termination of the exposure period. Standard clinical chemical analysis of blood was done at the end of the exposure period in all groups and in four males/dose group during the recovery period. Parameters measured were: blood urea nitrogen (BUN), alkaline phosphatase (AP), serum glutamine pyruvic transaminase (SGPT) and serum cholinesterase (ChE).

A number of toxic signs were observed in the animals exposed at 20 mg/kg/day. Both sexes had a marked decrease in body weight gain, with this decrease becoming significant by day 13 in the males and by day 20 in the females. A decrease in water consumption also was observed so that, by the end of the exposure period, the difference was statistically significant in the females and marginal in the males. Absolute body weights also were decreased in this dose group, as were organ weights for brain, liver, thymus, kidney and testis. Heart weight was significantly decreased in females, with a trend toward a decrease seen in the males. Organ-to-body weight ratios for heart, liver, brain and kidney were increased in both sexes receiving 20 mg/kg/day. A significant decrease in the organ-to-body weight ratio was reported for the thymus (females only) and testes.

Serum ChE was significantly decreased at sacrifice (93 days) in the females receiving 20 mg/kg/day. AP activity was increased (statistically significant, $P<0.05$) in these females at that time, but the investigators suggested that this change may not have been treatment-related. PCV, RBC and Hgb levels were all significantly decreased in the animals receiving 20 mg/kg/day on day 76 and at sacrifice.

In the group receiving 20 mg/kg/day, animals of both sexes had statistically significant increases in the landing foot-spread measurement by day 22. This became even more pronounced at day 29, at which time the test was discontinued so that the animals would not be injured. Peripheral nerve and spinal cord lesions were seen by light and electron microscopy in all rats given the 20 mg/kg dose. Electron microscopic observations made at 33 days of treatment in the males revealed myelin and axonal degeneration with focal loss of axons. At sacrifice, all females exhibited severe degeneration in the sciatic nerve (50% or more of all fibers affected). This degeneration was characterized by severe demyelinization, axonal degeneration and loss of axons. Males exhibited the same changes, half of them (5/10) at the severe level (>50% of the neurons affected), the other half at the moderate level (20% to 50% of the neurons affected). In addition, degenerative myelopathy was observed in the fasciculus gracilis of the spinal cord. This damage was observed in at least one of the three sections made through the cord (cervical, thoracic and lumbar levels) of 5/10 males and 9/10 females. The changes were characterized by demyelinization and swollen astrocytes and axons. The severity varied from equivocal to slight. No treatment-related lesions were observed at the light microscopic level in sections of the brain from the cerebrum, cerebellum or midbrain.

Other effects which were judged to be treatment-related in the animals treated with 20 mg/kg/day included skeletal muscle atrophy, especially of the hind limb (2/10 males, 8/10 females), slightly increased hematogenous pigmentation of the spleen (4/9 females), hyperkeratosis and ulcerative gastritis in the non-glandular stomach (4/10 males), atrophy of mesenteric fat (8/10 females), testicular atrophy (10/10 males), focal or multifocal mineralization in the seminiferous tubules (5/10 males), increased cellular debris and/or decreased spermatogonic elements in the tubular lumina of the epididymis (9/10 males), vacuolization of the smooth muscle of the wall of the urinary bladder (1/10 males, 2/9 females) and pulmonary inflammation (3/10 males, 5/10 females).

In animals treated with 5 mg/kg/day, no significant changes were observed at the end of the exposure period (92 or 93 days) in body weight gain, absolute body weight and relative and/or absolute organ weights, except for the liver in the males. Both the absolute organ weight and the liver-to-body weight ratio were increased significantly in the males. No effects were observed on water consumption, the landing foot-spread test or blood enzyme levels. Significant decreases in PCV, RBC and Hgb levels were observed in the females. Treatment-related effects in the peripheral nerves were observed histologically at the light microscopic level at this dose. The effects were of the same type as described for the highest dose, but they were generally less severe, varying from equivocal to very slight (focal or multifocal changes in individual neurons) in 9/10 males and 6/10 females. Ultrastructural alterations were also similar to those seen at the 20 mg/kg/day dose, although, again, less severe. Whereas about 55% of the fields tabulated in the 20 mg/kg group were affected, only about 34% of the fields were affected in the 5 mg/kg group.

No significant changes occurred in any non-neurological parameters measured in rats treated with the three lower doses (0.05, 0.2 or 1 mg/kg/day). No peripheral nerve lesions could be visualized at the light microscopic level. However, upon examination with the electron microscope, animals dosed at 1 mg/kg/day exhibited axolemmal invaginations in approximately 25% of the fields examined (about twice the control level). No significant alterations were observed at the two lowest dose levels. Therefore, on the basis of the most sensitive measure of toxicity (electron microscopic examination of peripheral nerves) in this study, a NOAEL of 0.2 mg/kg/day can be identified.

Satchell and McLeod (1981) observed the clinical effects following oral ingestion of acrylamide by greyhound dogs. Fourteen dogs (sex not stated) were given acrylamide in the diet (7 mg/kg/day) and examined daily. Toe scuffing, an early sign of peripheral neuropathy, occurred between 20 and 38 days of treatment. The mean accumulated dose at the onset of toe scuffing was 210 mg/kg (S.D. = ±40). The first signs of ataxia (limb crossing and walking with a rolling motion of the hind quarters), were seen after 28 to 53 days of treatment; the mean accumulated dose at the onset of ataxia was 290 mg/kg

(S.D. = ±50). Onset of muscle weakness occurred between 44 and 67 days of treatment. The mean accumulated dose of acrylamide was 390 mg/kg (S.D. = ±30). Toe folding was the last sign of sensory impairment, with onset between 48 and 66 days of treatment; the mean accumulated dose at that time was 410 mg/kg (S.D. = ±30). Feeding disturbance was observed late in the experiment. The onset of regurgitation, although difficult to identify precisely, ranged from 54 to 68 days of treatment. Radiological examination of one of the animals with suspected abnormal feeding behavior (after 63 days of acrylamide dosing) revealed the typical appearance of megaesophagus, as well as all the other signs of neuropathology. The etiological association of vagal neuropathology with megaesophagus suggested to the authors that an axonopathy of the vagus may occur in acrylamide poisoning.

Schaumburg et al. (1982) examined whether electrophysiologic changes preceded axonal degeneration in acrylamide-treated monkeys. Monkeys were injected daily with doses of 1, 2, 3 or 10 mg/kg of acrylamide. The earliest abnormality was detected at one to two weeks in animals dosed at 10 mg/kg. A latency shift in the onset of near-field brain stem somatosensory evoked potential (SEP) activity overlying the gracile nucleus was noted following unilateral electrical stimulation of the peripheral nerve. The SEP configuration gradually returned to normal after cessation of acrylamide treatment. Animals dosed at 1 mg/kg of acrylamide developed SEP abnormality only after 18 months of daily dosing. Non-invasive brainstem SEP thus provided a sensitive test for early detection of peripheral neuropathy of the distal-axonopathy type.

Maurissen and Weiss (1982) studied the effect of acrylamide on sensory thresholds in monkeys trained to report detection of vibratory and electrical stimuli. Acrylamide dissolved in water was mixed with fruit juice and administered orally at a dose of 10 mg/kg, five days/week (cumulative dose 320 to 450 mg/kg, average daily dose of 7.1 mg/kg). Animals were observed five days/week. Two times/week they were weighed and presented with a visuomotor task which consisted of retrieving small baits placed in recessed wells under a grid. Weight loss usually preceded the onset of gross behavioral disturbance such as loss of balance. Sensitivity to electrical stimulation was not clearly affected. Sensitivity to vibrational stimulation decreased during treatment and remained affected for several weeks after cessation of dosing.

Approximately six months after the last dose of acrylamide, three monkeys from the study described above were subjected to a repetition of the same experiment (Maurissen and Weiss 1984). They were dosed with acrylamide at 10 mg/kg, five days/week. Two monkeys served as controls. As in the previous experiment, electrical sensitivity was not impaired and vibration sensitivity was reduced in the treated monkeys. Analysis of covariance demonstrated that the rate of recovery was generally the same after the first and the second experiment.

Pryor et al. (1983) described neurobehavioral effects of acrylamide in adult male Fischer-344 rats. Acrylamide was administered orally five days/week for 15 weeks at doses of 0, 6.6, 9.6, 13.8 or 19.9 mg/kg (average daily doses of 0, 4.7, 6.9, 9.9 or 14.2 mg/kg). Toxicity was assessed at regular intervals by a battery of tests designed to detect toxicant-induced alterations in neuromotor function (grip strength), coordination (rotation orientation) and motor activity. Responsiveness to painful stimuli was assessed using a standard tail flick analgesic assay. Visual, auditory and proprioceptive functions were tested using a multisensory conditioned pole-climb avoidance procedure. Body weight and rectal temperature were recorded concurrently with the above tests. All rats dosed with 14.2 mg/kg/day died between week 7 and 15. The highest dose of acrylamide that was not lethal was 9.9 mg/kg/day. After 15 weeks of treatment at 9.9 mg/kg/day, a significant reduction in weight gain was observed. Recovery occurred within three weeks after cessation of dosing. Forelimb grip strength was affected significantly at this dose after 12 and 15 weeks of treatment. Recovery was complete three weeks after cessation of dosing. Hind limb grip strength was affected in a dose-related manner, being statistically significant in the groups receiving 9.9 and 6.9 mg/kg/day. These rats became progressively weaker and were slow to recover.

At the end of 15 weeks of dosing, the average hind limb grip strength of rats treated with 9.9 mg/kg/day of acrylamide was 27.3% of controls; six weeks after the end of dosing, this value was 73.8% of controls. The 9.9 mg/kg/day dose of acrylamide markedly impaired rotation orientation after 15 weeks of dosing, but recovery was rapid and complete three weeks after cessation of dosing. A significant difference in the acoustic startle effect was observed after 15 weeks of dosing at 9.9 mg/kg/day, and a greater difference in the reaction to the air-puff stimulus was observed. Startle to the air puff stimulus was also significant in the 6.9 mg/kg dosing group. Responses by treated rats to both stimuli were different than responses by control rats throughout the recovery phase. Only the 9.9 mg/kg/day dose of acrylamide impaired responses to all three stimuli of multi-sensory conditioned avoidance response (CAR). The CAR performance was still impaired markedly six weeks after cessation of dosing. At the 4.7 mg/kg/day concentration no significant effects were observed. There were no significant effects on motor activity, thermal sensitivity or rectal temperature at any dose level. Based upon the observation of several neurobehavioral parameters, a NOAEL of 4.7 mg/kg/day can be identified in this study.

A summary of these long-term studies of acrylamide toxicity is presented in Table V-6.

C. Other Effects

1. Carcinogenicity

The effect of acrylamide on tumor initiation in female Sencar mice was investigated by Bull et al. (1984). Six- to eight-week-old mice in groups of 40 were exposed to acrylamide by oral, intraperitoneal, or topical application. Acrylamide (>99% pure, judged by GC/MS analysis) was administered six times over two weeks at doses of 12.5, 25.0 or 50.0 mg/kg (average daily doses of 5.4, 10.7 or 21.4 mg/kg/day, respectively). The tumor promotion regimen was

Table V-6 Summary of Acrylamide Effects Following Long-Term Exposure

Animal species	Average daily dose (mg/kg)	Length of exposure	Effects observed	Reference
Cat	1	125 days	Ataxia	Kuperman (1958)
Rat	0.3-3	90 days	NOAEL	McCollister et al. (1964)
	7-11	189 days	NOAEL	
	30	21-90 days	Hind limb ataxia Death (5/5 males)	
	40	14-24 days	Hind limb ataxia	
Cat	0.02-0.2	360 days	NOAEL	McCollister et al. (1964)
	0.7	240 days	Hind limb weakness	
	2.1	68 days	Hind limb weakness	
	7.1	26 days	Hind limb weakness	
		40 days	Hind limb ataxia	
Monkey	0.02-0.71	363 days	NOAEL	McCollister et al. (1964)
	2.1	363 days	Sluggish reflexes	
	7.1	48-70 days	Hind limb weakness	
Rat	10-14	12-16 weeks	Hind leg weakness	Fullerton and Barnes (1966)
		6 months	Reduced nerve conduction velocity	
	15-18	4-10 weeks	Hind limb neuropathy	
	20-30	3 weeks	Hind limb neuropathy	
		2 months	Reduced nerve conduction velocity	
Baboon	10	42-97 days	Ataxia	Hopkins (1970)
		118 days	Wallerian degeneration	
	15	42 days	Ataxia	
	20	16 days	Ataxia	
Baboon	10	115 days	Reduced muscle action potential, recovery in 2-3 months	Hopkins and Gilliat (1971)
	15	94 days	Severe reduction in nerve and muscle action potentials, incomplete recovery after two years	

continued-

Table V-6 - continued

Animal species	Average daily dose (mg/kg)	Length of exposure	Effects observed	Reference
Cat	3	70 days 7 months 253-295 days	Gait disorder Rear limb weakness Demyelination	Schaumburg et al. (1974)
Rat	0.05-0.2	93 days	NOAEL	Burek et al. (1980)
	1	93 days	No effect on any non-neurologic parameter Increased axolemmal invaginations	
	5	93 days	No increase in foot splay, neuropathology by light and electron microscopy	
	20	13-93 days	Multiple neural and non-neural adverse effects	
Dog	7	20-38 days 28-53 days 44-67 days 48-66 days 54-68 days	Toe-scuffing Ataxia Muscle weakness Toe-folding Regurgitation, megaesophagus	Satchell and McLeod (1981)
Monkey	1	1[illegible] months	SEP alterations	Schaumburg et al. (1982)
	10	7-14 days	SEP alterations	
Monkey	7.1	6-9 weeks	Decreased sensitivity to vibrational stimulation, no effect on electrical stimulation	Maurissen and Weiss (1982)

continued-

Table V-6 - continued

Animal species	Average daily dose (mg/kg)	Length of exposure	Effects observed	Reference
Rat	4.7	15 weeks	NOAEL	Pryor et al. (1983)
	6.9	15 weeks	Decreased response to auditory and tactile stimuli	
	9.9	12-15 weeks	Decreased grip strength, decreased multisensory response	
	17	15 weeks	50% lethality	
	14.2	7-15 weeks	100% lethality	

begun two weeks after acrylamide treatment. A dose of 1 μg of 12-0-tetradecanoyl-phorbol-13-acetate (TPA) was applied in 0.2 mL acetone to the shaved back of each mouse, three times/week for 20 weeks (control mice, 20/group, received 0.2 mL acetone treatment at the same frequency and duration). Tumor incidence was charted from weekly observation. All animals were killed at 52 weeks and all gross lesions were evaluated histopathologically. There was a significant dose-dependent effect of acrylamide on time-to-first-tumor, as well as for appearance of multiple tumors ($P<0.01$). No tumors occurred in the absence of TPA promotion. Greater tumor yield was observed following oral administration of acrylamide than after intraperitoneal or topical application, as reflected by a significantly shorter time-to-first-tumor by the oral versus the topical ($P<0.01$) or intraperitoneal ($P<0.01$) routes, at equivalent doses. The yield of squamous cell papillomas and carcinomas as a function of route of exposure was: oral>intraperitoneal>topical (Table V-7).

Bull et al. (1984) also investigated the effect of acrylamide on the incidence of lung adenomas in male and female A/J mice following oral and intraperitoneal routes of administration. Groups of 40 male and 40 female mice, eight-weeks-old, were administered oral doses of 6.25, 12.5 or 25.0 mg/kg acrylamide (>99% pure) dissolved in distilled water, three times/week, for eight weeks (average daily doses of 2.7, 5.4 or 10.7 mg/kg/day, respectively). The animals were killed at nine months of age. Other groups of 16 males and 16 female mice, eight-weeks-old, received intraperitoneal injections of 1, 3, 10, 30 or 60 mg/kg (average daily doses of 0.43, 1.3, 4.3, 12.9, 25.7 mg/kg) of acrylamide dissolved in distilled water three times/week for eight weeks. These animals were killed at eight months of age. Lungs

Table V-7 Tumor-Bearing Animals and Classification of Histologically Examined Skin Tumors in Sencar Mice Initiated With Acrylamide by Various Routes

Cumulative dose[b] (mg/kg)	Route of administration	TPA	Cumulative number of tumor-bearing animals[a]/number of animals initiated	Number of squamous cell papillomas/number of animals examined	Number of squamous cell carcinomas/number of animals examined	Percentage of animals bearing squamous cell carcinomas
Acrylamide						
0[d]	p.o.	+	2/40	0/34[c]	0/34[c]	0
75	p.o.	+	12/40	3/35	2/35	5.7
150	p.o.	+	23/40	8/33	7/33	21.2
300	p.o.	+	30/40	11/38	6/38	15.8
300	p.o.	-	0/20	0/17	0/17	0
0[d]	i.p.	+	0/40	0/35	0/35	0
75	i.p.	+	10/40	2/38	2/38	5.2
150	i.p.	+	13/40	3/36	4/36	11.1
300	i.p.	+	21/40	6/35	4/35	11.4
300	i.p.	-	0/20	0/17	0/17	0
0[e]	Topical	+	7/40	5/36	0/36	0
75	Topical	+	4/40	3/38	1/38	2.6
150	Topical	+	11/40	3/35	2/35	5.7
300	Topical	+	18/40	2/34	3/34	8.8
300	Topical	-	0/20	0/20	0/20	0

[a] To be included in the cumulative count, the animals must have had a lesion 1 mm in diameter for three consecutive weekly observations.

[b] Six doses given over two weeks.

[c] Number of animals available for histological examination following death, or at termination of the experiment at one year. Number excludes heavily autolyzed and cannibalized animals.

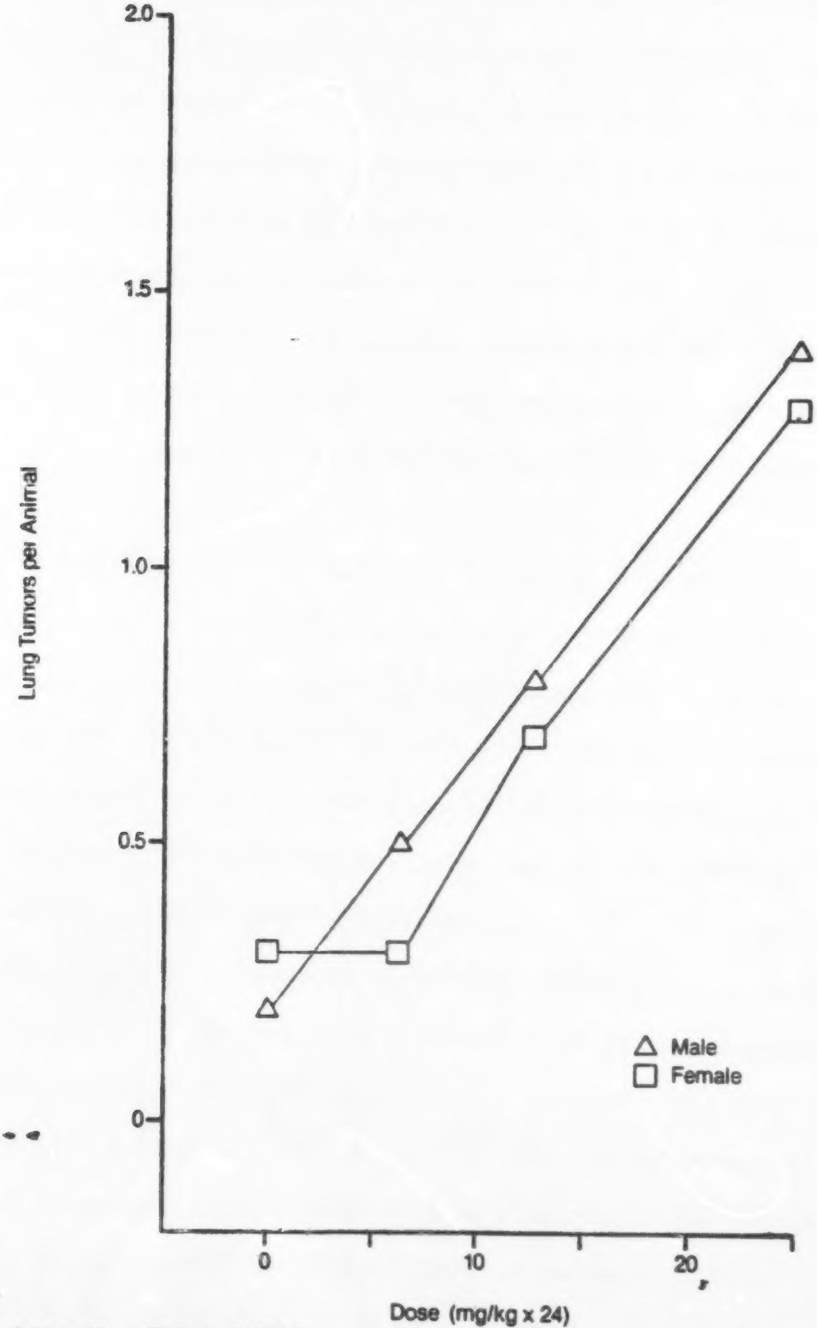
[d] Distilled deionized water was administered in the same volume (0.2 mL/mouse) and frequency as used in experimental groups.

[e] Ethanol was administered topically in the same volume (0.2 mL/mouse) and frequency as used for experimental groups.

Adapted from Bull et al. (1984).

were removed at necropsy, fixed, and the number of surface adenomas were counted. Acrylamide administered by the oral route produced an increase in the yield of lung adenomas in both males and females in a dose-dependent manner (Figure V-1). The dose-response relationship was significant ($P<0.01$) when tested using a logit regression model analysis. Acrylamide administered by the intraperitoneal route also increased lung adenomas. Again the dose-relationship was statistically significant ($P<0.01$). Experiments with the 60 mg/kg dose were terminated after 11 injections because of frank peripheral neuropathy and decreased survival of these animals.

In another study groups of male and female Fisher 344 rats received drinking water containing acrylamide monomer at 0, 0.01, 0.1, 0.5 or 2.0 mg/kg/day for up to two years (Johnson, et al. 1984). After a year, significant depression of body weight was observed in the highest dose males. Distal neuropathy was observed in the peripheral nerves of animals in this group. Tumor incidence was not increased significantly in the groups receiving 0.01 or 0.1 mg/kg/day. Male rats receiving 0.5 mg/kg/day had significantly increased incidences of scrotal mesothelioma. Statistically significant increased incidences of tumors in the following tissues were determined in rats treated at 20 mg/kg/day: Females - mammary glands (benign and malignant), central nervous system (malignant), thyroid gland follicular epithelium (benign and malignant), mouth (benign), uterus (malignant) and clitoral gland (benign); Males - scrotal mesothelioma (malignant) and thyroid gland follicular epithelium (benign).



Adapted from Bull et al. (1984).

Figure V-1 Effect of Acrylamide Administered Orally on Development of Lung Tumors in A/J Mice

2. Mutagenicity

The mutagenic potential of acrylamide was tested by Ames assay (Bull et al. 1984). *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 were used. Dose levels of acrylamide ranging from 0.001 to 3.0 mg/plate were used in an initial assay and 3.0 to 30 mg in a repeat assay. Assays were performed with or without metabolic activation by the addition of liver microsomes from Arochlor-induced male Sprague-Dawley rats. Acrylamide failed to show an effect in any of the *Salmonella* strains with or without the addition of microsomes.

Miller et al. (1984), using the hepatocyte primary culture (HPC)-DNA repair test, found a lack of genotoxicity of acrylamide. Hepatocytes were isolated from male Fischer-344 rats and exposed to acrylamide and tritiated thymidine for 18 hours. Incorporation of radiolabel was determined by autoradiography. No DNA repair was observed at acrylamide concentrations up to 1×10^{-2} M. Higher doses were cytotoxic. Preliminary studies also indicated that acrylamide does not inhibit UV-induced DNA repair processes, and so the negative effect of acrylamide on thymidine incorporation noted above is probably not due to inhibition of repair enzymes.

Shiraishi (1978) reported on chromosomal aberrations in bone marrow and germ cells in mice following oral (Experiment I) or subcutaneous (Experiment II) administration of acrylamide. Sister-chromatid exchange studies also were reported. In Experiment I, four-week-old male DDY mice, weighing approximately 25 g, were fed a commercial powder diet containing 500 ppm (about 75 mg/kg) acrylamide for one, two or three weeks. Control groups were fed powder diet only. Cells undergoing mitosis were arrested in metaphase by injecting (intraperitoneally) 0.15 mL colchicine at a concentration of 1 mg/mL, two hours before killing the animals. Treated animals and controls were killed at the end of the dietary administration. In Experiment II, male DDY mice were injected (intraperitoneally) with a single dose of 50, 100, 150 or 200 mg/kg acrylamide in distilled water (intraperitoneal LD_{50} in DDY mice for acrylamide was 200 mg/kg). In each experiment, bone marrow and spermatogonia were sampled at 12 and 24 hours, 11 and 12 days after treatment.

Marrow cells in both experiments had no appreciable increase in frequency of chromosome aberrations in the exposed groups. However, there was a decrease in mitotic frequency which was so extreme in Experiment II at the 150 and 200 mg/kg doses, that observations could not be made at 11 to 12 days. Aneuploid and polyploid cells were frequently observed in cells from treated animals in both experiments. In spermatogonia, the dietary dose of acrylamide for three weeks (Experiment I) induced a significant increase in frequency of chromosome breaks when compared with controls ($P<0.01$) and the frequency of chromosomal aberrations was also increased significantly at two weeks ($P<0.05$). No significant effects were seen in spermatogonia from animals treated for one week. A striking reduction in the number of mitotic cells was observed in spermatogonia 12 and 24 hours after injection of 100 mg/kg acrylamide. With so few dividing cells remaining, it was impossible to examine for aberrations properly. At 50 mg/kg, no significant changes were observed.

The mitotic reduction was less remarkable in bone marrow, indicating that spermatogonia are more sensitive than bone marrow cells. Reduced mitosis and increased formation of aneuploid and polyploid cells suggested to the author that acrylamide damages cytoplasmic microtubules and the spindle. Chromosome rearrangements induced by acrylamide consisted of chain quadrivalents, ring quadrivalents, fragments and univalents in primary spermatocytes following both oral and intraperitoneal treatment. The sister-chromatid exchange frequency showed no noticeable increase over the control level in the bone marrow or the spermatogonial cells in Experiments I or II. Acrylamide also caused a significant reduction in testes weight, suggesting possible cell killing. Thus, the data show that acrylamide induced chromosome aberrations in the primary spermatocytes of mice, but caused no significant aberration in bone marrow cells.

3. Reproductive/Teratogenic Effects

Edwards (1976) studied the embryotoxic and teratogenic effects of acrylamide in the rat. Pregnant rats of the Porton strain were given acrylamide either as powder in the diet or by intravenous injection. In Group I, eight rats were given 200 mg/kg acrylamide in the diet (about 20 mg/kg/day) from the day of mating until parturition. In Group II, six rats were given 400 ppm (about 40 mg/kg/day) acrylamide in the diet from the day of mating for 20 days, and then were subjected to Caesarean section. In Group III, four rats were given a single intravenous dose of acrylamide (100 mg/kg) on day 9 of gestation. The only difference between the control and Group II was a

The mitotic reduction was less remarkable in bone marrow, indicating that spermatogonia are more sensitive than bone marrow cells. Reduced mitosis and increased formation of aneuploid and polyploid cells suggested to the author that acrylamide damages cytoplasmic microtubules and the spindle. Chromosome rearrangements induced by acrylamide consisted of chain quadrivalents, ring quadrivalents, fragments and univalents in primary spermatocytes following both oral and intraperitoneal treatment. The sister-chromatid exchange frequency showed no noticeable increase over the control level in the bone marrow or the spermatogonial cells in Experiments I or II. Acrylamide also caused a significant reduction in testes weight, suggesting possible cell killing. Thus, the data show that acrylamide induced chromosome aberrations in the primary spermatocytes of mice, but caused no significant aberration in bone marrow cells.

3. Reproductive/Teratogenic Effects

Edwards (1976) studied the embryotoxic and teratogenic effects of acrylamide in the rat. Pregnant rats of the Porton strain were given acrylamide either as powder in the diet or by intravenous injection. In Group I, eight rats were given 200 mg/kg acrylamide in the diet (about 20 mg/kg/day) from the day of mating until parturition. In Group II, six rats were given 400 ppm (about 40 mg/kg/day) acrylamide in the diet from the day of mating for 20 days, and then were subjected to Caesarean section. In Group III, four rats were given a single intravenous dose of acrylamide (100 mg/kg) on day 9 of gestation. The only difference between the control and Group II was a slight decrease in fetal weight of the latter which might be attributed to the lower maternal food intake of the treated animals. No macroscopic abnormalities of organ or skeletal structure were seen in fetuses from Group II. The litters in Groups I and III gained weight normally until weaning, and no gait abnormalities were observed. No abnormalities were found in the brains, spinal cords or sciatic nerves of these rats at six weeks of age, either macroscopically or by light microscopy.

The effect of acrylamide, administered by gavage to pregnant rats during gestation, on the developing dopamine system in the brain was investigated by Agrawal and Squibb (1981). Thirty-four time-bred female rats, Fischer-344 strain (approximately eight weeks old) were used. On day 7 of gestation, the animals were divided into two groups: Group I animals were dosed with acrylamide (20 mg/kg) daily for ten days by gavage. Group II served as the vehicle-dosed control. The mean body weight of acrylamide-dosed pregnant rats was never significantly different from control. No significant differences in birth weights or number of pups per litter were observed. Therefore, maternal toxicity from acrylamide exposure was considered to be absent. On day 1 after parturition, offspring were selected randomly from each litter to yield four experimental groups, with four males and four females in each. The four groups represented all combinations of fostering and cross-fostering with control and treated dams. On days 14 and 21, a male and female from each group were killed by decapitation and subjected to neurochemical study. There was significantly reduced ^{3}H-spiroperidol binding in the striatal tissue of two-week-old pups (males and/or females) in all groups (except treated mother with control pups) relative to control mother with control pups. There were no significant differences in ^{3}H-spiroperidol binding in three-week-old pups. This suggests that the effect is reversible. The effect of gestational exposure to acrylamide appears to be opposite to that seen in adults, where acrylamide causes an increase in the endogenous dopamine level and number of dopamine binding sites. These results suggest that early exposure to acrylamide may temporarily affect the developing dopaminergic system.

Walden et al. (1981) monitored the concentrations of selected enzymes involved in intestinal metabolism in rats exposed to acrylamide *in utero* and during lactation. Thirty-four time-bred Fischer-344 pregnant rats were separated into two groups after six days of gestation. Group I rats were dosed daily for ten days (until day 16) by gavage (20 mg acrylamide/kg, dissolved in water). Group II rats were given equal volumes of water and served as controls. There were no overt signs of maternal toxicity throughout this study. Litter size, sex ratio and number of pups were unaffected by exposure to acrylamide. On the day of birth, the pups and mothers were cross-fostered to form the following groups: treated dams with pups from treated dams (T-T), treated dams with control pups (T-C), control dams with pups from treated dams (C-T), and control dams with control pups (C-C). Cross-fostering permitted distinction between effects resulting from prenatal and lactational exposure. On days 14, 21 and 60 after birth pups from each group were killed (without regard to sex) for the determination of intestinal levels of acid phosphatase (AcP), alkaline phosphatase (AP), β-glucuronidase (BG), citrate synthetase (CS) and lactate dehydrogenase (LDH). On day 24 after the pups were weaned, the mothers were sacrificed for measurement of the same intestinal enzyme activities.

Prenatal and lactational exposure to acrylamide significantly influenced the concentration of several intestinal enzymes in the pups during early stages of development. Alkaline phosphatase (AP) activities were significantly increased at 14 and 21 days, and significantly decreased at 60 days in the C-T group as compared to the C-C group, indicating a prenatal effect. A significant increase of AP at 14 days and a significant decrease at 60 days in the T-C group as compared to the C-C group indicated a lactational effect. A significant decrease in AP activity at 14 and 21 days and a significant increase at 60 days in the T-T group as compared to the C-T group showed an enhancement effect. The activity of citrate synthetase was not affected in any manner in any of the experimental groups. The activity of LDH was significantly increased at 21 days for the T-C group as compared to the C-C group, indicating a lactational effect. The activity of AcP was significantly increased at 14 days and decreased at 60 days in the C-T group, as compared to the C-C group, showing a prenatal effect. Also, a significant decrease at 21 days and an increase at 60 days was found in the T-T group, as compared to the C-T group, indicating enhancement of prenatal effects. Activity of BG was increased at 21 days in the C-T and T-C groups, indicating prenatal and lactational effects. Also, a decrease in BG activity was observed at 14 and 21 days in the T-T group indicating enhancement of prenatal effect. The authors concluded that both prenatal and postnatal exposure significantly changed intestinal enzyme levels during early development.

Ali et al. (1983) studied the effect of acrylamide on the levels of circulating testosterone, growth hormone and prolactin in male Fischer-344 rats. Animals were injected intraperitoneally, once per day for 20 days, with an aqueous solution of acrylamide at doses of 0, 10 or 20 mg/kg. Twenty-four hours after the last treatment, the animals were killed by decapitation and hormones were assayed by radioimmunoassay of serum. A dose-dependent depression of testosterone and prolactin concentrations was found which was significant at the 20 mg/kg dose ($P<0.05$). Growth hormone concentration was not significantly different from control values at either treatment dose.

The toxic effect of acrylamide and several related compounds on the testis was investigated by Hashimoto et al. (1981). Male mice (DDY strain, five- to six-weeks of age, weighing about 29 g at the beginning of the experiment) were distributed randomly into groups of five to seven animals. Groups were dosed orally with test compounds, twice weekly for 8 to 10 weeks, at a concentration of 35.5 mg/kg (average daily dose of 10.1 mg/kg/day). In order to examine the effect of metabolic activation, mice were injected intraperitoneally with phenobarbital (PB) at a dose of 50 mg/kg for five successive days per week, from one week prior to the start of the study until the last week of the study. Four neurotoxic compounds (acrylamide, N-hydroxymethylacrylamide, N-isopropylacrylamide and N-methylacrylamide) and one non-neurotoxic compound, (N,N'-methylene-bis-acrylamide) produced both testicular atrophy and significant reduction in testes weight, with degeneration of the epithelial cells of the seminiferous tubules. The interstitial cells were normal. Another neurotoxic compound (methacrylamide) did not produce any toxic effect on the testes, suggesting that neurotoxicity and testicular toxicity may occur by different mechanisms. The effects of phenobarbital (PB) pretreatment on acrylamide and acrylamide-analog testicular toxicity are shown in Table V-8. The authors concluded that (1) testicular damage was completely prevented by PB in mice treated with acrylamide or N-isopropylacrylamide, (2) testicular damage was reduced by PB in mice treated with acrylamide or N-isopropylacrylamide and (3) testicular damage was reduced by PB in mice treated with other analogues. The authors deduced that acrylamide and N-isopropylacrylamide are converted to inactive compounds in mice and that PB may enhance this conversion.

D. Summary

Acrylamide is toxic to experimental animals. Most estimates of the LD_{50} for a single oral dose are between 150 and 250 mg/kg. The toxic effects of acrylamide are cumulative, and LD_{50} values for multiple exposures range from 15 to 50 mg/kg/day, depending on duration of exposure.

Sub-lethal dosing regimens cause many adverse health effects in animals. Exposures ranging from 9.9 mg/kg/day for 15 weeks to 50 mg/kg/day for nine days have been observed to cause reduced food and water consumption, decreased weight gain or net weight loss and altered organ weights.

Most adverse health effects of acrylamide appear to be the result of damage to central or peripheral nervous tissue. The most characteristic effects are weakness and ataxia in hind limbs, progressing to paralysis with

Table V-8 Effect of Acrylamide and Analogs on the Body and Testicular Weights in Mice, and the Effect of Metabolic Activation

Compound	Number of mice	Dose[a] mmol/kg (LD_{50})	Duration (weeks)	Body weight[b] (g)	Relative testicular weight[c]
		No metabolic activation			
Control (0.9% saline)	6	--	8	34.5±2.2	0.36±0.05
Acrylamide	6	0.5 (1/3)	8	32.4±2.4	0.30±0.02[d]
N-hydroxy-methylacrylamide	5	2.9 (1/2)	8	36.5±6.3	0.20±0.05[e]
N-isopropyl-acrylamide	6	1.2 (1/3)	8	37.0±4.3	0.28±0.04[d]
N-methylacrylamide	7	1.9 (1/3)	8	40.0±3.8	0.22±0.05[e]
N,N'-methylene-bis-acrylamide	5	1.3 (1/2)	8	34.8±2.9	0.17±0.03[e]
		After metabolic activation with phenobarbital[f]			
Control (0.9% saline)	7	--	10	36.0±2.7	0.34±0.04
Acrylamide	7	0.5 (1/3)	10	37.1±2.5	0.31±0.02
N-hydroxymethyl-acrylamide	6	2.9 (1/2)	10	33.5±2.3	0.26±0.03[c]
N-isopropyl-acrylamide	6	1.2 (1/3)	10	36.1±3.4	0.31±0.07
N-methyl-acrylamide	6	1.9 (1/3)	10	34.9±4.5	0.25±0.07[d]

[a] Oral dose twice weekly.
[b] Mean ± SD.
[c] Testis weight/body weight x 100
[d] $P<0.05$.
[e] $P<0.01$.
[f] Sodium phenobarbital 50 mg/kg, 5 times/week.

Adapted from Hashimoto et al. (1981).

continued exposure. More sophisticated tests of nerve damage (microscopic integrity, rate of axonal transport) often are positive before development of the external signs noted above. Due to the strong cumulative toxicity of acrylamide, the size of the daily dose required to produce nerve damage decreases as a function of increasing exposure periods.

Few studies of neurotoxocity following a single dose of acrylamide have been reported. In rats, retrograde transport in nerve was significantly decreased 12 hours after single intraperitoneal injections of doses of 35 mg/kg or higher, while doses of 15 mg/kg or lower did not reduce retrograde transport rate.

When acrylamide was given for periods from one to four weeks, daily doses of 5 to 30 mg/kg generally produced adverse effects. Cats receiving 10 mg/kg/day had no histological signs of neurotoxicity by 11 days, but developed external signs of neuropathy by 17 to 22 days. Cats receiving 30 mg/kg/day had depressed tendon reflexes in five days, and gait disorders by ten days. Dogs receiving 5 mg/kg/day developed ataxia by 21 days, and demyelination of nerves was evident after 60 days. Rats receiving 15 mg/kg/day had reduced axonal transport by five days and increased foot- splay by ten days. Rats receiving 30 mg/kg/day for 14 to 21 days demonstrated hind limb splaying and neuropathology by microscopic examination of nerves, while rats receiving 1 to 3 mg/kg/day for 21 days had no signs of neurotoxicity. Monkeys treated with an average dose of 7.1 mg/kg/day developed signs of impaired vision in 28 days (apparently from damage to central nerves), while ataxia and motor impairment did not occur until 46 to 65 days of exposure.

Signs of acrylamide toxicity in animals exposed for longer periods of time (several months to one year) are generally the same as in animals exposed for shorter times, but average daily doses as low as 1 mg/kg/day sometimes produce effects. In one experiment, cats receiving 1 mg/kg/day for 125 days developed ataxia, and similar results were obtained in another experiment where hind limb weakness occurred in cats receiving 0.7 mg/kg/day for 240 days. In rats receiving 1 mg/kg/day for 93 days there were no external signs of toxicity, but histologic evidence of neuropathology was noted. The NOAEL in this study was 0.2 mg/kg/day. Monkeys receiving 1 mg/kg/day for 18 months had altered somatosensory function. Some studies found effects only at higher daily doses. For example, rats receiving up to 11 mg/kg/day for 189 days had no external signs of toxicity, and monkeys receiving 2.1 mg/kg/day for 363 days developed only slightly sluggish reflexes. Doses of 5 to 30 mg/kg/day produce numerous signs of toxicity within a few weeks to several months.

Recent results indicate acrylamide may be a carcinogen. Female rats receiving 2 mg/kg/day for up to 18 months had increased incidence of several tumor types. Male rats receiving a dose of 0.5 mg/kg/day for two years had an increased frequency of scrotal tumors, and animals receiving a dose of 2 mg/kg/day for two years had increased frequency of scrotal, thyroid and adrenal tumors. Mice exposed to average daily doses of 5.4, 10.7 or 21.4 mg/kg/day for two weeks had a dose-dependent increase in tumors induced by the phorbol ester TPA. Mice exposed to average daily doses of 2.7, 5.4 or 10.7 mg/kg/day for eight weeks showed an increased incidence of lung adenomas.

Acrylamide did not elicit mutagenic activity in the Ames test (with or without microsomal activation) or in the hepatocyte primary culture DNA repair test. Chromosome aberrations were noted in spermatogonia of mice exposed to 75 mg/kg/day for two to three weeks, but marrow cells were not affected. Both marrow cells and spermatogonia had a striking decrease in mitotic index following a single dose of 50 to 150 mg/kg.

Treatment of pregnant rats with 20 to 40 mg/kg/day during gestation did not result in any external or histological signs of toxicity in the pups. A more sensitive test (^{3}H-spiroperidol binding in brain) indicated that 20 mg/kg/day did affect the fetuses. Assay of intestinal enzymes of pups from mothers receiving 20 mg/kg/day revealed evidence of both prenatal and lactational effects.

Acrylamide at doses of 10 to 20 mg/kg/day for 20 days depressed testosterone levels in male mice, and doses of 10 mg/kg/day for eight to ten weeks caused testicular atrophy.

VI. HEALTH EFFECT IN HUMANS

A. Clinical Case Studies

Igisu et al. (1975) described the health effects of acrylamide in a Japanese family of five individuals that was exposed through ingestion and external use of well water contaminated with acrylamide seeping from a grouting operation. The well water was analyzed by gas chromatography and found to contain 400 mg/L acrylamide and a trace of dimethylaminopropionitrile. Assuming a daily water consumption of 2 L and a body weight of 70 kg for the adults, the exposure corresponds to a daily dose of about 11.4 mg/kg. Symptoms of toxicity developed about a month after the grouting operation and the mother, father and grandmother were hospitalized. Each exhibited similar symptoms, consisting of marked rhinorrhea, coughing, dizziness, irrational behavior, poor orientation and memory, as well as visual, auditory and tactile hallucinations followed by slurred speech and unsteadiness in walking. Both women had ecchymoses of the extremities. Retention of urine and feces was noted in the mother only. Hematology, blood chemistry and liver function were normal. Electroencephalography suggested a pattern of excessive sleepiness. The mother had no deep tendon response and all three patients exhibited slowed sensory nerve conduction velocities. The two children, who were at school much of the time and apparently did not consume as much well water as the adults, had mild gait and sleep disturbances. Both children recovered within two weeks, and the adults recovered within two to four months.

Other reports on human exposure to acrylamide described dermal and/or inhalation exposure, with most occurring among occupationally exposed workers. Auld and Bedwell (1967) described acrylamide poisoning in a 21-year-old white male, whose job in a grouting operation involved loading hoppers with 10% acrylamide solution. This individual was not careful in handling the solution and splashed the liquid on his face and forearms. He developed a reddish rash on his forearms after working for approximately two weeks. After working seven weeks, he noted weakness in his legs, and at nine weeks he started stumbling when he walked, had poor balance and experienced difficulty in writing and handling eating utensils because of muscular weakness. Subsequent symptoms included profuse sweating of arms and legs and numbness and tenderness in hands and feet. He was hospitalized 14 weeks after his first exposure. Neurologic examination revealed an absence of tendon reflexes and impairment of position and vibration senses. Elevated cerebrospinal fluid protein content was the only abnormality revealed by standard clinical analysis of body fluids. Recovery was achieved after 14 weeks of hospitalization.

Garland and Patterson (1967) described acrylamide poisoning in six men (19, 23, 30, 56, 57 and 59 years old) who had worked for periods varying from 4 to 60 weeks in factories manufacturing acrylamide-based flocculants. Exposure to acrylamide occurred via the skin and to some extent via the ingestion of dust. The exact quantity of acrylamide to which these workers were exposed and the duration of exposure were not mentioned. Typical signs and symptoms presented by these men included increased sweating of hands and feet, fatigue, muscle weakness (most marked in lower limbs), erythema and peeling of hand skin, sensory loss, absence of reflexes and unsteadiness with loss of balance. Generalized tremors, slurring of speech, weight loss and some bladder disturbance were also observed in some of the men. Individuals with relatively mild symptoms recovered completely within 2 to 12 months after exposure to acrylamide ceased. Severely affected persons did not appear to recover completely following cessation of exposure. The clinical picture in these individuals suggested peripheral neuropathy and mid-brain disturbance.

Fullerton (1969) made further electrophysiologic and histologic observations on three of the severely affected acrylamide-exposed workers (19, 56 and 59 years old) described above (Garland and Patterson 1967). Maximal motor nerve conduction in the digital ends of median and ulnar nerves was found to be within the normal range; however, values were elevated in the right lateral nerve of one worker. Response to nerve stimulation in small muscles was dispersed irregularly. In two workers, no ascending action potentials could be recorded from the median nerve at the wrist following stimulation of index finger digital nerves, and a diminished action potential was recorded in the third worker. These results indicated an involvement of peripheral sensory fibers. Examination of a biopsy sample of sural nerve of one person (exposed to acrylamide for 2.5 months) revealed fibers undergoing Wallerian-type degeneration. A quantitative study on transverse sections of sural nerve showed a reduction in the large diameter fibers. Since nerve degeneration and regeneration occurred during or immediately after acrylamide exposure, Fullerton suspected that nerve function was impaired before symptoms of acrylamide intoxication developed.

Davenport et al. (1976) presented a detailed clinical study of severe peripheral neuropathy in a 25-year-old man who handled acrylamide in a factory for six months. His work involved mixing raw acrylamide powder with other dry and liquid reagents in a sealed reactor vessel. Neither the dose nor the duration of exposure to acrylamide was mentioned. Although he used personal protection devices, the man experienced intense irritation and ulceration of his palms and soles shortly after beginning the job. During the next three months, he became anorectic and lost 40 pounds of body weight. Subsequently he developed unsteady gait. Although the man stopped working, he developed progressively worsening ataxia. Subsequently, tingling in the hands, slurred speech and difficulty in swallowing developed. Terminal dysmetria and intention tremor of the upper extremities and a moderate truncal and gait ataxia with wide base were observed. Sensory appreciation of pinprick and temperature was impaired below the midcalf and midforearm with raised threshold for light touch in the same areas. Position and vibration senses were absent below the knees and the Romberg test was positive. Deep tendon and plantar reflexes could not be elicited. Eye movements were conjugate and full with fine sustained nystagmus on lateral gaze. Palatal and pharyngeal functions were normal, although there was a mild speech impairment. Serum creatine phosphokinase and serum glutamic oxaloacetic transaminase concentrations were elevated. Electromyographic examination revealed bilateral denervation potentials in both the gastrocnemius and tibialis anterior muscles.

A portion of the sural nerve was resected at the level of the lateral malleolus and processed for light and electron microscopic study. Diffuse fibrosis and loss of fibers were observed by light microscopy. Several enlarged axons, both myelinated and non-myelinated, were present. Adequately stained sections showed evidence of focal axonal enlargements. Focal dilation of the myelin sheath was commonly observed in teased fibers. Electron microscopy revealed numerous axons with bundles of fine filaments, some of which were oriented haphazardly. In these regions, very few neurotubules could be identified, and there was an increase in the number of small mitochondria and dense bodies. Fibers undergoing Wallerian degeneration were also present. Two months after the period of exposure, there was neither progression nor regression in the patient's symptoms.

A small epidemic of acrylamide poisoning among construction workers was reported by Kesson et al. (1977). The work involved the use of acrylamide monomer which was polymerized in the confines of a small concrete tunnel. The workers did not take adequate safety precautions and were subjected to acrylamide vapors as well as splashing and drenching of clothing from acrylamide solution. Two of the men (47 and 57 years old) experienced peeling of skin, sweating, muscle weakness with absent tendon reflexes, wasting of the small muscles of both hands and patchy sensory impairment in both arms. These men had not completely recovered after more than one year. Four other men (26 to 38 years old) had increased sweating and peeling of skin and three of these had sensory loss in their hands. They did recover fully.

The National Institute for Occupational Safety and Health document on acrylamide discussed in detail the toxic effects of exposure of humans to acrylamide in occupational settings (NIOSH 1976). These reports deal with qualitative aspects of clinical signs and symptoms of acrylamide intoxication, and no quantitative data are presented on the concentration to which the persons were exposed or the blood and tissue levels of acrylamide in the workers. Nevertheless, a review of the information indicates that exposure to acrylamide produces a consistent toxicity profile in humans which depends on the duration of exposure. Early signs and symptoms of acrylamide poisoning in humans are tenderness in fingers, numbness in lower limbs, excessive perspiration of the limbs, desquamation of the hands and muscular weakness of hands and feet. Prolonged exposure causes body weight loss, apathy and hypersomnolence, as well as emotional disturbance, positive Romberg's sign, loss of proprioceptive sensations (vibration and position), weakened tendon reflexes, and various motor difficulties (ataxic gait, foot drop, muscle atrophy). Retention of urine and feces may also occur. Neurological defects are noted in both the peripheral and central nervous systems. An extensive and critical overview of acrylamide neurotoxicity was presented by Tilson (1981). Spencer and Schaumburg (1974a) also have reviewed human exposure to acrylamide.

B. Epidemiologic Studies

No reports dealing with epidemiologic studies of acrylamide were found in published literature.

C. High Risk Subpopulations

No published reports were found which identify any subpopulation at greater risk than the general population to the adverse health effects produced by acrylamide exposure.

D. Summary

Only one report of human acrylamide intoxication via ingestion of drinking water was found in the literature. In this case, a family of five members (three adults and two children) used water from a well for drinking, cooking, washing and bathing. Analysis of the well water revealed the presence of 400 mg acrylamide/L. The adults were most affected, and required hospitalization. All three adults exhibited similar signs of widespread central and peripheral nervous system dysfunction. The two children were less affected, probably because they were at school much of the time and were less exposed. All five members recovered fully.

Numerous other reports of human exposure to acrylamide have appeared, generally dealing with dermal or inhalation exposure of workers under occupational conditions. Quantitative information regarding the dose and duration of such exposures was usually lacking, but a similar profile of adverse health effects was often observed. As with oral ingestion, many of the signs reflect dysfunctions of either the central or peripheral nervous systems.

No reports dealing with the epidemiology of acrylamide were found in the published literature. No population at high-risk to acrylamide exposure has been identified in the published literature.

VII. MECHANISM OF TOXICITY

Acrylamide enters cells freely and is retained in many tissues of the body (see Section III.E.) but many of the adverse health effects of acrylamide exposure are consequences of disturbed function in central or peripheral nerves. For this reason, studies regarding the mechanism of acrylamide toxicity have mainly focused on effects in neural tissue. Results indicate that acrylamide may interfere with several aspects of nerve cell function, including (1) inhibition of important enzyme activities, (2) decreased protein synthetic capacity, (3) decreased axonal transport and (4) decreased levels of neurotransmitters and/or receptors.

A. Effects on Enzyme Activities

Howland et al. (1980b) investigated the inhibitory effect of acrylamide *in vitro* on enzyme activities in extracts of rat brain. Whole brains from male Sprague-Dawley rats (180 to 200 g) were homogenized and a 1,200 x g supernatant fraction was prepared for measurement of glyceraldehyde phosphate dehydrogenase (GAPDH), lactate dehydrogenase (LDH) and phosphofructokinase (PFK). Enolase was measured in a 100,000 x g supernatant. The effect of acrylamide was assessed after incubating the extracts with acrylamide at 37° C for 20 min (GAPDH, LDH, PFK) or 30 min (enolase). Acrylamide (3 mM) increased the K_m and decreased the V_{max} of enolase. This inhibitory effect could be entirely reversed by dialysis of the extract following acrylamide exposure. Dithiothreitol (DTT), at concentrations from 2 mM to 8 mM caused a dose-dependent increase in acrylamide inhibition of enolase, and similar results were observed with glutathione (GSH, 2 mM to 4 mM). The inhibitory effect of acrylamide plus DTT (100 mM) could not be reversed by dialysis. The authors proposed that the unexpected potentiating effects of DTT and GSH were due to reduction of disulfide bonds present in enolase which could then react (irreversibly) with acrylamide. Acrylamide treatment of extracts also resulted in inhibition of GAPDH (I_{50} about 0.4 mM) and PFK (maximum of about 64% inhibition at 15 mM acrylamide), but LDH activity was not affected by incubation with 20 mM acrylamide.

These studies were extended by Howland et al. (1980a). As above, acrylamide was found to inhibit enolase activity in brain extracts *in vitro* (I_{50} = 3.7 mM). The effect of acrylamide administered *in vivo* by intraperitoneal injection (50 mg/kg/day for 11 days, total dose of 550 mg/kg) was assessed on total enolase and nerve-specific enolase activity in nerve and brain. In sciatic nerve, acrylamide treatment reduced total enolase activity only slightly (not statistically significant, $P>0.05$), but reduced nerve-specific enolase activity to undetectable levels. In brain, acrylamide treatment had no significant effect on total enolase, but reduced nerve-specific enolase by 39% ($P<0.05$). The authors suggested that inhibition of nerve-specific enolase may be an important factor in development of acrylamide-induced neuropathy.

Spencer et al. (1979) found that acrylamide inhibited GAPDH and PFK enzyme activity *in vitro*. Lactate dehydrogenase (LDH), an enzyme not directly involved in the production of energy via the citric acid cycle, was not affected by acrylamide.

In vitro addition of dithiothreitol protected the thiol groups of GAPDH and PFK, preventing the effect of acrylamide on these enzymes.

The effect of acrylamide on pyridine nucleotide metabolism in rats was tested by Johnson and Murphy (1977). Acrylamide was administered in aqueous solution intraperitoneally at an initial dose of 4 mg/kg/day to male Holtzman rats (260 to 300 g). When no neurologic symptoms became apparent after one week, the dosage was increased to 40 mg/kg/day and the animals were killed when the cumulative dose was either 428 mg/kg (low dose) or 668 mg/kg (high dose). The impairment of neurological function was tested by the vertical rack test (an animal was scored successful in this test if it remained attached to the rack in two of three trials). Interference with pyridine nucleotide metabolism was tested by measuring concentrations of oxidized pyridine nucleotides in brain extracts. Impairment of pyridine nucleotide function was evaluated in three ways. First, substrates of pyridine nucleotide-dependent enzymes in both quick-frozen brains and in brains subjected to periods of ischemia were measured. Second, concentrations of high-energy phosphate sources and the rates of their use during ischemia were compared. Third, the redox states of cytoplasmic NAD^+ and $NADP^+$ were determined. The concentration of NAD^+ was significantly increased ($P<0.01$) in brains from high dose animals. The concentrations of α-glycerophosphate was also significantly elevated in the brains of high dose animals ($P<0.05$). The survey of other metabolites and energy use showed no other difference between experimental and control groups. These observations suggested that phospholipid metabolism may be affected by acrylamide in view of the fact that

NAD^+ is known to be a stimulator of phospholipid synthesis and that α-glycerophosphate provides the glycerol backbone for phospholipids.

Kaplan and Murphy (1972) studied the effects of acrylamide on sciatic nerve β-glucuronidase (BG) activity in male Holtzman rats (200 to 300 g). Aqueous solutions of acrylamide were administered intraperitoneally, at a dose of 50 mg/kg/day for eight days. A slight but significant ($P<0.05$) rise in sciatic nerve BG activity was observed one week after the last dose of acrylamide. The enzyme activity continued to increase, reaching a peak of 340% of control values 30 days after the last dose. The ability of animals to maintain their balance on a rotating rod ("rotarod") was taken as an index of neuromuscular status. Rotarod performance of acrylamide-treated rats revealed that the neuromuscular impairment was maximum at days 6 through 11 and recovery from the effect was almost complete by day 15 of the experiment. The authors suggested that the increase in BG activity reflected peripheral nerve regeneration. In a separate experiment it was observed that rats exposed to acrylamide for eight days (as above) were signficantly ($P<0.01$) more susceptible to a second acrylamide exposure (50 mg/kg/day) on day 30 (when BG activity in the sciatic nerve was 320% of control) than previously untreated animals. The mean time-to-failure for rotarod performance in acrylamide re-treated rats was 2.5 days as compared to four days in previously untreated rats. Return of BG to near normal levels at 90 days was associated with a return of acrylamide-treated animals to normal sensitivity to acrylamide retreatment.

B. Effects on Protein Synthesis

Hashimoto and Ando (1973) studied the effect of acrylamide on protein synthesis in nerve cells by measuring incorporation of ^{14}C-lysine and ^{35}S-methionine into neural tissue *in vitro*. Male Sprague-Dawley rats were fed a diet containing 500 ppm acrylamide (about 25 mg/kg/day, assuming a body weight of 300 g and food consumption of 15 g/day) for four weeks. The animals displayed hind limb weakness after two weeks, slight ataxia after three weeks and paralysis after four weeks. Animals were sacrificed at various times during and after exposure. Samples of brain, spinal cord, sciatic nerve and liver tissue slices were taken and incubated with radioactive amino acids. A complex pattern of effects involving both decreased and increased label incorporation was observed. Specifically, in sciatic nerve, ^{14}C-lysine incorporation was significantly decreased at two weeks, and became significantly increased at six and eight weeks (two and four weeks after exposure ceased). Incorporation of ^{35}S-methionine into sciatic nerve was not biphasic, but was increased at four and six weeks. In spinal cord, increased incorporation of ^{35}S-methionine was seen at four weeks, and increased incorporation of both ^{14}C-lysine and ^{35}S-methionine were observed at six weeks. No significant change in incorporation of either label into brain or liver was noted. The authors interpreted these data as indicating a preferential sensitivity of sciatic nerve and spinal cord to acrylamide, and suggested that the increased incorporation of label into these tissues may be due to nerve regeneration.

Schotman et al. (1977) studied the effect of acrylamide on protein synthesis by measuring incorporation of amino acid into tissue proteins *in vivo*. Female Wistar rats (150 to 170 g) were injected intraperitoneally with ^{3}H-leucine, and decapitated 20 minutes later. Sections of cervical, thoracic and lumbar spinal cord, brain stem and heart muscle were removed, and the amount of acid-insoluble radioactivity measured. In rats that had been treated with four doses of acrylamide (50 mg/kg, one dose per two days, cumulative dose of 200 mg/kg) no sensory or motor neuronal deficits were observed. However, a significant decrease in the incorporation of radioactivity, ranging from 11.7% to 13.5% ($P<0.05$), was observed in all three spinal cord segments and in brain stem. In animals that had been treated with doses of 20 mg/kg/day for 22 days (cumulative dose of 440 mg/kg), ataxia and minor changes in terminal motor nerves were noted and the incorporation of radiolabel was decreased more than in the previously described group (17.5% to 22.7%, $P<0.05$). There was also a similar decrease in incorporation of label into heart muscle ($P<0.05$), indicating that the effect of acrylamide was not unique to nerve tissue.

Animals that received seven doses of acrylamide at 50 mg/kg/2 days (cumulative dose of 350 mg/kg) developed ataxia, but recovered completely by 17 days after exposure ceased. In these animals an increase in the incorporation of radiolabel of about 11% was observed relative to controls ($P<0.05$). There was no difference between control and exposed animals in the uptake of label by heart muscle during this recovery phase.

These investigators also studied the effect of acrylamide on the incorporation of ^{14}C-leucine into tissue slices of cervical spinal cord *in vitro*. This effect was studied by the addition of 10^{-5}, 10^{-4} or 10^{-3} M acrylamide to the medium used for incubation of slices. No changes in the incorporation of label were noticed between control and treated slices. Similar results were obtained when cervical spinal cord slices obtained from rats treated with acrylamide (50 mg/kg/day for eight days) were used. This indicated that acrylamide toxicity *in vivo* may develop slowly or may be mediated by a metabolite of acrylamide.

In conclusion, the authors noted that inhibition of label incorporation is not necessarily related to a change in protein synthesis, but might be due to changes in amino acid pools in the tissue. Nevertheless, the correlation between the levels of the *in vivo* incorporation rate and the progressive state of the neuropathy suggested a relation between a disturbance in protein metabolism and acrylamide-induced neuropathy.

C. Effects on Axonal Transport

Pleasure et al. (1969) studied the effect of acrylamide on flow rates of axonal proteins in motor and sensory nerves of cats. Acrylamide was administered orally (20 mg/kg/day, five days/week). In about two weeks, distal axonal degeneration was found in nerves of the hind limb. No morphological alterations in the cell bodies of motor or sensory neurons were evident at this time. Flow rates of axonal proteins in motor and sensory nerves were determined by injecting ^{3}H-leucine intraperitoneally and measuring over time the appearance of radioactivity in a segment of the lumbosacral cord (complete with roots and ganglion). The results indicated that in normal cats axonal proteins move from lumbosacral cord along the ventral roots at about 1.6 mm/day, and from ganglion cells toward the spinal cord along the dorsal roots at about 1.3 mm/day. In seven of nine cats treated with acrylamide, radioactivity accumulated in segments of roots close to the cell bodies of origin, and did not appear along ventral and dorsal roots. This suggested that protein synthesis occurred in the cell bodies, but that there was an abnormality in the protein transportation mechanism within the axons. (In two cats, killed three and seven days after receiving leucine, the distribution of label in the ventral root was similar to that seen in the controls.)

Sumner et al. (1976) measured axoplasmic transport in cats with dying back type of polyneuropathy induced by daily injections of acrylamide (10 mg/kg) for 16 to 99 days. Tritiated L-proline was injected into the L-7 dorsal root ganglion and six hours later the fast axoplasmic transport was measured. The mean velocity of the front of radioactivity in seven treated animals was 399 mm/day (±SD 18.39) and in five control animals was 436 mm/day (±SD 26.08). The difference was statistically significant ($P<0.01$). Slowing axoplasmic flow was observed before the onset of clinical signs of neuropathy and no further slowing of axoplasmic transport was observed as animals developed advanced neuropathy. Slow axoplasmic transport was measured using similar techniques 30 days after injection of ^{3}H-proline, and no difference was noted between control and acrylamide-treated animals.

Sahenk and Mendell (1981) studied transport of proteins in sciatic nerve following acrylamide-induced axonopathy. Male Sprague-Dawley rats (160-180 g) were given daily intraperitoneal injections of an aqueous solution of acrylamide (40 mg/kg) for 16 to 19 days. Treated rats developed ataxia with moderate hind-limb paralysis. By special surgical procedures, L5-S1 ventral horn segments of the spinal cord were injected at multiple sites with tritiated leucine. These animals were killed at sequential five hour intervals between 5 to 40 hours after injections of label. Three hours before sacrifice, two ligatures were placed 10 mm apart on the sciatic nerve. At the time of killing, 5 cm segments of sciatic nerve were removed and the retrograde transport of labeled material was determined by measuring the accumulation of radioactivity in the 2 mm segment immediately below the distal collection ligature. Orthograde transport was determined by measuring radioactive accumulation in the 2 mm segment immediately above the proximal ligature. In acrylamide-treated rats, the accumulation of radioactivity at the distal ligature was somewhat delayed and moderately reduced in magnitude. Analysis of the data indicated a decrease in bi-directional transport rate within the distal axons, rather than a failure in the turn-around process in the nerve terminal.

The effect of acrylamide treatment on retrograde axoplasmic flow was studied by Miller et al. (1983). The researchers injected ^{125}I-labeled nerve growth factor (NGF) into the front foot pad of male Sprague-Dawley rats (200 g) and measured the appearance of ^{125}I in dorsal root ganglia (C_4 to C_7) 4 to 24 hours later. Acrylamide was administered by intraperitoneal injection 15 minutes after ^{125}I-NGF injection. Doses of 25 to 100 mg/kg inhibited retrograde transport in a dose-dependent manner when measured 12 hours later. A single dose of 15 mg/kg did not significantly affect retrograde flow. In animals that received daily intraperitoneal acrylamide injections (15 mg/kg/day) prior to injection of ^{125}I-NGF, retrograde transport was inhibited after five days (cumulative dose of 75 mg/kg). Changes in retrograde transport were detected prior to detectable alterations in peripheral nerve function.

The effect of acrylamide on retrograde transport in neurons was further studied by Miller and Spencer (1984). Experimental animals were male CD-1 mice (20 to 25 g). Purified iodinated tetanus toxin (TTX) was used as the transport marker. The velocity of retrograde transport was assessed by determining the position of the leading edge of transported TTX in axons at times following single doses of acrylamide. Acrylamide at doses up to 100 mg/kg produced a dose dependent decrease in transport of TTX to the perikarya of sensory neurons in dorsal root ganglia and in motor neurons in the anterior horn. Acrylamide was a more potent inhibitor of retrograde transport in sensory axons than in motor axons. Acrylamide reduced the velocity of TTX transport by up to 75%. (No change was detected in neuronal uptake of TTX.) The authors concluded that acrylamide produced profound alterations in retrograde transport which preceded functional or morphologic neuropathy.

D. Effects on Neurotransmitters and Receptors

Dixit et al. (1980) studied the effect of acrylamide, alone and in combination with diethyl maleate (DEM, a known glutathione depletor), on the concentration of biogenic amines in the brain. Young adult female rats (100 g) received daily intraperitoneal injections of either saline (controls), acrylamide only (50 mg/kg in saline), DEM only (1.25 μmol/kg in peanut oil), or DEM followed 30 minutes later by acrylamide. After nine days, all animals treated with both DEM and acrylamide had developed hind-limb paralysis, while only 40% of the animals treated with acrylamide only showed this sign. At this time animals were killed and the concentrations of dopamine, noradrenaline and serotonin were measured in homogenates of whole brain. The results are shown in Table VII-1. Acrylamide (alone) significantly decreased the concentrations of all three neurotransmitters, and concentrations were further decreased significantly by the combined treatment (DEM followed by acrylamide).

These studies were extended by Dixit et al. (1981). Male Wistar rats (125 g) received acrylamide (25 mg/kg/day) by gastric intubation for 21 consecutive days. After seven days of treatment, no effect on body weight, brain weight or nerve function was observed. Neurotransmitter concentrations in whole brain homogenate were also unchanged. After 14 days, convulsions and mild ataxia had developed, and complete hind-limb paralysis was present by day 21. These signs of neuropathy were accompanied by significant decreases ($P<0.01$ to 0.02) in noradrenaline, dopamine and serotonin concentrations in brain homogenates on day 14 and day 21.

In addition, Dixit et al. (1981) further characterized the effects of acrylamide on neurotransmitter levels in specific regions of the brain. Male

Table VII-1 Effect of Acrylamide and Diethyl Maleate (DEM) on Brain Neurotransmitter Concentrations.

Transmitter (mg/g brain)	Control	DEM	Acrylamide	DEM + Acrylamide
Noradrenaline	0.35±0.04[a]	0.34±0.03	0.25±0.01[b]	0.19±0.01[b]
Dopamine	0.72±0.02	1.02±0.14	0.60±0.07[b]	0.42±0.03[b]
Serotonin	1.80±0.12	1.44±0.19	1.46±0.06[b]	1.05±0.05[b]

[a] Values are means ± S.D., n=4.
[b] $P<0.05$.

Adapted from Dixit et al. (1980).

Wistar rats (125 g) were given acrylamide (50 mg/kg/day) by gastric intubation for five days. The concentrations of noradrenaline, dopamine and serotonin (5-HT) in various regions of the brain are shown in Table VII-2. Acrylamide treatment resulted in significant decreases in noradrenaline concentrations in the pons medulla and the basal ganglia. Dopamine levels were significantly reduced in the cerebellum, pons medulla and hypothalamus. Serotonin content was significantly decreased in pons medulla, hypothalamus and cerebral cortex.

Agrawal et al. (1981a) studied the effect of acrylamide on neurotransmitter receptors in various brain regions. Six-week-old male Fischer rats were administered by gavage a single oral dose of acrylamide (0, 25, 50 or 100 mg/kg) dissolved in water (six to eight animals per dose). The rats were killed 24 hours later and specific brain regions were dissected out and prepared for binding studies. The final concentrations of radioactive ligands, the competing unlabeled compounds used to determine non-specific binding and the receptor sites assayed are summarized in Table VII-3. The effect of acrylamide on cerebral receptors 24 hours after dosing with acrylamide are presented in Table VII-4. Significant increases in ^{3}H-spiroperidol binding were observed in the striatum of rats that received acrylamide at all three dose levels ($P<0.05$). Significant increases in glycine receptors in the medulla and in serotonin receptors in the frontal cortex were observed in rats that received 100 mg/kg. No changes were found in muscarinic receptors in the striatum, benzodiazepine receptors in the frontal cortex, or gamma aminobutyric acid (GABA) receptors in the cerebellum. Thus, the enhanced dopamine receptor binding was a specific effect of acrylamide toxicity. Pretreatment

Table VII-2 Effect of Acrylamide (50 mg/kg) Treatment in Rats on Regional NA, DA and 5-HT Concentrations of Brain[a]

Regions	NA (µg/g)		DA (µg/g)		5-HT (µg/g)	
	Control	Acrylamide	Control	Acrylamide	Control	Acrylamide
Cerebellum	0.18±0.02	0.17±0.02	1.55±0.06	1.10±0.05[b] (29.0)	1.55±0.15	1.29±0.14
Pons medulla	0.78±0.02	0.53±0.01[b] (32.4)	2.78±0.20	1.95±0.20[d] (29.6)	2.42±0.17	1.39±0.16[c] (42.6)
Midbrain	0.76±0.07	0.60±0.06 (21.0)	2.32±0.21	1.95±0.10[e] (16.07)	2.80±0.14	2.85±0.12
Hypothalamus	2.63±0.24	2.61±0.23	10.6±0.5	7.77±0.02[b] (27.0)	2.81±0.14	2.00±0.12[c] (32.0)
Basal ganglia	1.66±0.10	1.00±0.09[c] (40)	1.13±0.05	0.90±0.05	1.91±0.20	1.92±0.18
Cerebral cortex	0.29±0.22	0.23±0.04	1.62±0.06	1.50±0.05	1.56±0.12	1.238±0.11[c] (25)

[a] Data given in parentheses show percentage change. Mean ± SE for five rats.
NA = Noradrenaline, DA = Dopamine and 5-HT = 5-Hydroxytryptamine (serotonin).

[b] $P<0.001$.

[c] $P<0.01$

[d] $P<0.05$.

[e] N.S.

Adapted from Dixit et al. (1981).

Table VII-3 Pharmacological Agents Used in Cerebral Receptor Studies

Labeled ligand	Specific activity (Ci/mmol)	Conc. (nM)	Unlabeled competitor	Conc. (µM)	Receptor species assayed
(1-Phenyl-4^{3}H) spiroperidol	23	1.0	Haloperidol	1.0	Dopamine
DL-(Benzilic-4,4'-^{3}H) quinuclidinyl benzilate	29.4	1.0	Atropine	1.0	Acetylcholine
(Methylene-^{3}H)-diazepam	73	0.7	Diazepam	1.0	Benzodiazepine
(Methylene-^{3}H (N))-muscimol	7.3	8.0	GABA	1.0	GABA
(G-^{3}H)-Strychnine sulfate	13	6.0	Strychnine	10.0	Glycine
(1,2-^{3}H (N))-Serotonin	29.8	3.1	Serotonin	1.0	Serotonin

Adapted from Agrawal et al. (1981a).

Table VII-4 Effect of a Single Dose of Acrylamide on Cerebral Receptors

Receptor	Region	Dose of acrylamide (mg/kg)			
		0	25	50	100
Dopamine	Striatum	334±13	413±11[a]	417±11[a]	481±32[a]
Muscarinic cholinergic	Striatum	527±26	490±25	479±21	549±33
Benzodiazepine	Frontal cortex	76±6	79±6	63±5	80±4
GABA	Cerebellum	640±64	768±48	576±48	544±32
Glycine	Medulla	558±96	636±24	654±42	690±35[a]
5-HT	Frontal cortex	66±5	72±5	70±4	84±7[a]

Binding expressed as pmol/g protein ± S.E.

[a] Differing from zero dose ($P<0.05$, Fisher's Least Significant Difference Test).

Adapted from Agrawal et al. (1981a).

of animals with SKF 525A (a blocker of hepatic mixed function oxidase), or with methylmercury chloride (a thiol blocker), prevented the acrylamide-induced increase in striatal ^{3}H-spiroperidol binding. This indicated that the causative agent may be a metabolite of acrylamide, rather than the parent compound.

In a similar study Agrawal et al. (1981b) treated six-week-old male Sprague-Dawley rats with single doses of acrylamide (0, 50, 100 or 200 mg/kg), administered by gavage. Striatal tissue was dissected out 24 hours after treatment. A significant increase ($P<0.05$) relative to control values in ^{3}H-spiroperidol binding was noticed at all doses of acrylamide. The affinity of binding of receptors from treated animals was greater than that of controls. However, there was no significant change in striatal dopamine levels in treated animals. Increased binding of ^{3}H-spiroperidol was also seen 24 hours after ten repeated daily doses of 10, 20 or 30 mg/kg/day. Normal binding values were restored within eight days after cessation of dosing, indicating that the effect of acrylamide on central neurotransmitter receptors is reversible.

Agrawal and Squibb (1981) studied the effects of acrylamide administration to pregnant Fischer-344 rats on dopamine receptor binding in pups after birth (details of this study are presented in Section V.C.3., reproductive/teratogenic effects). Acrylamide was administered to pregnant females daily from day 7 through 16 of gestation. Significant reduction in ^{3}H-spiroperidol binding in the striatal tissue of two-week-old pups was observed. This effect was not seen in pups after three weeks, and thus was reversible. This effect of gestational exposure on dopamine receptor binding is opposite to that seen in adult rats (where acrylamide exposure causes increased endogenous dopamine levels and increased number of dopamine receptor sites).

Bondy et al. (1981) studied neurotransmitter receptors in brain regions of acrylamide-treated rats following repeated doses of acrylamide. Six-week-old male Fischer rats were administered acrylamide by gavage (10 treatments spread over a two-week period) at doses of 0, 5, 10 or 20 mg/kg. At the end of treatment, the rats were killed by decapitation, their brains removed and brain regions dissected out. Receptor binding studies were carried out with membrane fractions. Twenty-four hours after the last dose, significant increases in striatal dopamine and acetylcholine binding were observed at both doses tested ($P<0.05$). Significant increases in GABA binding in the cerebellum, glycine binding in the medulla and serotonin binding in the frontal cortex were observed only at the highest dose (20 mg/kg/day). No change was found in the benzodiazepine receptor in the frontal cortex. One week after the final acrylamide dose, the degree of binding for all ligands studied was similar to controls, indicating that the effects were reversible.

E. Synergistic/Antagonistic Effects

The potentiating effect of diethyl maleate (DEM, a glutathione depletor) on acrylamide-induced hind-limb paralysis and depletion of brain neurotransmitters has been discussed above (Section VII.D.).

Edwards (1975b) investigated a series of compounds with chemical structures related to acrylamide, with regard to their neurotoxicity alone and their interaction with acrylamide. The compounds investigated are listed in Table VII-5. Male Porton rats (200 g) were fed these analogues in the diet at the doses shown in Table VII-5, with or without intraperitoneal injection of acrylamide (50 mg/kg, two to three times per week). N-hydroxymethyl acrylamide, N,N-diethyl acrylamide and N-methyl acrylamide all produced signs of neuropathy when given alone, and enhanced the neurotoxicity of acrylamide. It was not clear whether these effects were additive or synergistic. A number of acrylamide-related compounds were not neurotoxic and did not enhance acrylamide neurotoxicity.

Loeb and Anderson (1981) found that vitamin B_6 may partially protect against acrylamide-induced neurotoxicity. Male Sprague-Dawley rats (230 to 290 g) received daily intraperitoneal injections of saline (controls), acrylamide (30 mg/kg/day), vitamin B_6 (5 mg/rat/day), or both acrylamide and vitamin B_6. Using the inclined screen test, signs of acrylamide-induced neurotoxicity were significantly ($P<0.05$) delayed in onset and reduced in magnitude in animals also receiving vitamin B_6. Treatment with vitamin B_6 did not significantly reduce acrylamide-induced neurotoxicity as judged by the foot-spread test, nor did it protect against acrylamide-induced weight loss.

Kaplan et al. (1973) studied the effects of hepatic microsomal enzyme inducers on acrylamide-induced neuropathy in male Holtzman rats (200 to 300 g). Sodium phenobarbital (50 mg/kg/day) was administered for 20 days.

Table VII-5 Summary of Neurotoxicity of Compounds and of Their Additive Effects with Acrylamide

Compound	Formula	Average dose (mg/kg)	Reactivity with glutathione[a]	Produces neurotoxicity	Enhancement of acrylamide neuropathology
Acrylamide	CH_2:$CHCONH_2$	14 to 21	0.91	+	+
Ethyl crotonate	CH_3CH:$COOC_2H_5$	1,865	0.24	-	-
Methyl methacrylate	CH_2:$C(CH_3)COOCH_3$	2,050	0.17	-	-
Methylene-bis-acrylamide	$(CH_2$:$CHCONH)_2CH_2$	105 to 115	0.54	-	-
N,N'-pentamethylene-acrylamide	CH_2:$CHCONC_6H_5$	170	Not Tested	-	-
N,N'-bis-acrylamido-acetic acid	$(CH_2$:$CHCONH_2)CHCOOH$	110	Not Tested	-	Not Tested
3,3-Iminodipropionamide	$NH(CH_2CH_2CONH_2)_2$	57[b]	Not Tested	-	-
Glutathione-S-propionamide	Glutathione-$SCH_2CH_2CONH_2$	243[b]	Not Tested	-	Not Tested

continued-

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[a] Values given as the rate constant ($l\ mol^{-1}\ min^{-1}$). Compounds were tested for neurotoxicity by appraisal of gait disorder. + = positive result, - = negative result.

[b] Administered by intraperitoneal injection.

Table VII- 5 continued

Compound	Formula	Average dose (mg/kg)	Reactivity with glutathione[a]	Produces neurotoxicity	Enhancement of acrylamide neuropathology
N-Hydroxymethyl-acrylamide	$CH_2:CHCONHCH_2OH$	93 to 135	0.91	+	+
N,N-Diethyl-acrylamide	$CH_2:CHCON(C_2H_5)_2$	195	0.058	+	+
N-Methylacrylamide	$CH_2:CHCONHCH_3$	95	0.058	+	+

[a] Values given as the rate constant ($l\ mol^{-1}\ min^{-1}$). Compounds were tested for neurotoxicity by appraisal of gait disorder. + = positive result, - = negative result.

[b] Administered by intraperitoneal injection.

Adapted from Edwards (1975b).

Beginning on day 6, acrylamide (40 mg/kg/day) was administered intraperitoneally. Another group of rats was treated with a single dose of dithiothreitol (200 mg/kg), five days prior to acrylamide administration as above. Neuronal integrity was assessed by rotarod performance. The total cumulative dose of acrylamide required to cause 100% failure of rotarod performance was 520 and 600 mg/kg respectively for dithiothreitol and phenobarbital-pretreated animals, as compared to 360 mg/kg for controls. Hepatic *in vitro* metabolism of acrylamide was studied in 9,000 x g supernatants from liver homogenates. Greater loss of acrylamide was detected in homogenates from phenobarbital-pretreated rat livers than from control livers. Phenobarbital-pretreated adult rats were observed histologically to have more peripheral nerve damage at the time of onset of functional impairment relative to that observed in non-phenobarbital treated animals.

Edwards (1975b) studied the effects of DDT and phenobarbital on acrylamide-induced neurotoxicity in male Porton rats (200 g). One group of rats received a single intraperitoneal injection of DDT (200 mg/kg bw) in corn oil five days before being given a diet containing 500 ppm acrylamide. Another group of rats received daily intraperitoneal injections of phenobarbital (50 mg/kg bw) for nine days, then five times/week throughout the rest of the experiment. On day six of phenobarbital dosing, these rats were put on a diet containing acrylamide (500 ppm). A control group was given acrylamide (500 ppm) only. After eight to ten days on the acrylamide diet, animals in all three groups developed slight ataxia. All animals recovered at the same time after cessation of acrylamide treatment. The results obtained in this study were thus different from that of Kaplan et al. (1973). Although the calculated daily dose of acrylamide was similar in both studies, acrylamide was given intraperitoneally in the Kaplan study whereas in the Edwards study, it was given in the diet. Also, for assessment of neurotoxic effect, Kaplan et al. (1973) used rotarod performance (a more precise measure), and Edwards (1975b) used the subjective analysis of hind limb paralysis.

F. Summary

Many of the adverse health effects of acrylamide are consequences of central or peripheral neurotoxicity. A number of effects of acrylamide on nerves which may contribute to or cause nerve dysfunction have been identified.

The activities of several important metabolic enzymes from the brain are reduced by incubation with acrylamide (1 to 10 mM) *in vitro*. Treatment of rats with acrylamide (50 mg/kg/day for 11 days) resulted in a specific reduction of nerve-specific enolase in sciatic nerve and brain. Increased concentrations of NAD^+ and α-glycerol phosphate were found in extracts of brain from rats treated with acrylamide (40 mg/kg/day for 16 days), suggesting an impairment in lipid metabolism. The activity of β-glucuronidase is increased in sciatic nerve following acrylamide exposure, but this may be a reflection of nerve regeneration.

Biosynthesis of proteins *in vivo* was decreased in spinal cord and brain in rats treated with four doses of acrylamide (50 mg/kg), even though no neuropathy was apparent. Higher doses of acrylamide produced neuropathy and further diminished protein synthesis. The inhibitory effect on protein synthesis was absent 17 days after exposure ceased. No effect of acrylamide (10^{-3} M to 10^{-5} M) on protein synthesis *in vitro* could be detected in spinal cord slices, suggesting that the effect may develop slowly *in vivo*. Biosynthesis of proteins in slices of sciatic nerve from rats treated with acrylamide (about 2.5 mg/kg/day for four weeks) was first decreased and then increased. Protein synthesis in liver and brain was not measurably affected, indicating a specific effect.

A number of studies have shown that acrylamide interferes with axonal transport. Inhibition of axonal transport in motor and sensory nerves was noted in cats receiving 20 mg/kg/day for two weeks. Fast axoplasmic flow was inhibited in dorsal root ganglia in cats receiving 10 mg/kg/day for 16 to 99 days. Slow axoplasmic flow was not changed by this treatment. Retrograde transport was diminished in sciatic nerve in rats receiving 40 mg/kg acrylamide for 16 to 19 days, in sensory and motor axons of mice receiving 25 to 100 mg/kg (single dose) and in dorsal root ganglia of rats that had received 15 mg/kg/day for five days. Changes in axonal transport were detected before the appearance of neuropathy.

Acrylamide also affects the concentrations of neurotransmitters and receptors in nerve tissue. Rats treated with acrylamide (50 mg/kg) for nine days exhibited significant reductions in the concentration of noradrenalin, dopamine and serotonin in the brain. In rats treated with 25 mg/kg/day, appearance of neuropathologic signs after 14 days correlated with significant decreases in brain neurotransmitter concentrations. Binding of a number of neurotransmitters is significantly increased in brain tissue from rats following treatment with a single dose of acrylamide (100 mg/kg). Ten oral doses of 10 to 30 mg/kg/day increased dopamine and acetylcholine receptors in striatal tissue. This effect was absent eight days after exposure ceased. Exposure of pregnant female rats to acrylamide resulted in decreased spiroperidol binding in two-week-old pups, but this was not observed in three-week-old pups.

A number of chemicals have been found to influence acrylamide toxicity. Diethyl maleate (DEM) increased acrylamide toxicity, presumably by acting to decrease cellular levels of glutathione. Vitamin B_6 supplementation provided a modest reduction in acrylamide-induced neuropathy by one test but not by another. Several derivatives of acrylamide, which are themselves neurotoxic, increased neurotoxicity when given together with acrylamide, but it was not clear if the effects were additive or synergistic. Pretreatment of rats with chemicals, such as phenobarbital or DDT, which induce hepatic microsomal enzymes, resulted in increased acrylamide neurotoxicity in one study, but not in another.

VIII. QUANTIFICATION OF TOXICOLOGICAL EFFECTS

The quantification of toxicological effects of a chemical consists of an assessment of the non-carcinogenic and carcinogenic effects. In the quantification of non-carcinogenic effects, an Acceptable Daily Intake (ADI) is calculated. From this an Adjusted Acceptable Daily Intake (AADI) and Health Advisory (HA) values for the chemical are then calculated to define the appropriate drinking water concentrations to limit human exposure. For ingestion data, this approach is illustrated as follows:

$$\text{ADI} = \frac{(\text{NOAEL or LOAEL in mg/kg/day})(\text{Body Weight in kg})}{\text{Uncertainty/Safety Factor}} = \text{mg/day}$$

$$\text{AADI} = \frac{\text{ADI}}{\text{Drinking Water Volume in L/day}} = \text{mg/L}$$

where:

NOAEL = no-observed-adverse-effect level.

LOAEL = lowest-observed-adverse-effect level.

Body weight = 70 kg for adult or 10 kg for child.

Drinking water volume = 2 L per day for adults or 1 L per day for children.

Uncertainty/Safety Factor = 10, 100 or 1,000.

Utilizing these equations, the following drinking water concentrations are developed for non-carcinogenic effects:

1. A one-day HA for 10-kg child.
2. A one-day HA for 70-kg adult.
3. A ten-day HA for 10-kg child.
4. A ten-day HA for 70-kg adult.
5. A lifetime AADI for a 70-kg adult.

The distinctions made between the HA calculations (items 1 through 4) are associated with the duration of anticipated exposure. Items 1 and 2 assume a single acute exposure to the chemical. Items 3 and 4 assume a limited period of exposure (possibly 1 to 2 weeks). The HA values will not be used in establishing a drinking water standard for the chemical. Rather, they will be used as informal scientific guidance to municipalities and other organizations when emergency spills or contamination situations occur. The AADI value (item 5) is intended to provide the scientific basis for establishing a drinking water standard based upon non-carcinogenic effects.

A NOAEL or LOAEL is determined from animal toxicity data or human effects data. For animal data, this level is divided by an uncertainty factor because there is no universally acceptable quantitative method to extrapolate from animals to humans. The possibility must be considered that humans are more sensitive to the toxic effects of chemicals than are animals. For human data, an uncertainty factor is also used to account for the heterogeneity of the human population in which persons exhibit differing sensitivity to toxic chemicals. An Office of Drinking Water (ODW) modification of the guidelines set forth by the National Academy of Sciences (NAS 1977, 1980) is typically used in establishing uncertainty factors as follows:

- An uncertainty factor of 10 is used when good acute or chronic human exposure data identifying a NOEL/NOAEL are available and are supported by acute or chronic toxicity data in other species.

- An uncertainty factor of 100 is used either when good acute or chronic toxicity data identifying NOEL/NOAEL are available for one or more animal species (but human data are not available), or when good acute or chronic toxicity data identifying a LOAEL in humans are available.

- An uncertainty factor of 1,000 is used either when limited or incomplete acute or chronic toxicity data are available, or when good acute or chronic toxicity data identify a LOAEL (but not a NOEL/NOAEL) for one or more animal species.

The uncertainty factor used for a specific risk assessment is judgmental. Factors that cannot be incorporated in the NAS/ODW guidelines for selection of an uncertainty factor, but must be considered include: (1) the quality of the toxicology data, (2) the significance of the adverse effect and (3) the existence of counterbalancing beneficial effects.

If toxicological evidence requires the chemical to be classified as a potential carcinogen (there is evidence to classify acrylamide as a carcinogen following ingestion), mathematical models are used to calculate the estimated excess cancer risks associated with the ingestion of the chemical via drinking water. The bioassay data used in these estimates are from animal experiments.

In order to predict the risk for humans from these data, it must be converted to an equivalent human dose. This conversion includes correction for non-continuous animal feeding, non-lifetime studies and for the difference in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average human body weight is 70 kg and that the average human consumes 2 liters of water per day. The multistage model is then fit to the equivalent human data to estimate the risk at low doses. The upper 95% confidence limit of this estimate is used. Excess cancer risks can also be estimated using other models such as the one-hit model, the Weibull model, the logit model and the probit model. There is no basis in the current understanding of the biological mechanisms involved in cancer to choose among these models. The estimates of low doses for these models can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of risk rate levels has an inherent uncertainty. This is because the tools of scientific measurement, by their very nature, involve both systematic and random error. In most cases, only studies using experimental animals have been performed. There is thus uncertainty when the data are extrapolated to humans. When developing risk rate levels, several other areas of uncertainty exist, such as (1) incomplete knowledge concerning the health effects of contaminants in drinking water, (2) the impact of test animal age, sex and species and the nature of target organ systems examined on the toxicity study results and (3) the actual rate of exposure of internal targets in test animals or humans. Dose-response data are usually only available for high levels of exposure, not for the lower levels of exposure for which a standard is being set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

A. Non-Carcinogenic Effects

1. Lethality

Several studies have estimated the LD_{50} of acrylamide in experimental animals. Following administration of a single oral dose, McCollister et al. (1964) found LD_{50} values ranging from 150 mg/kg to 180 mg/kg in rats, guinea pigs and rabbits. In rats, NIOSH (1976) determined the single oral dose LD_{50} to be 203 to 277 mg/kg and Pryor et al. (1983) obtained a value of 203 mg/kg.

The toxicity of acrylamide tends to be cumulative, and LD_{50} values following sub-acute or sub-chronic exposures are lower than for a single dose. Pryor et al. (1983) measured an oral LD_{50} in rats of 32 mg/kg/day following subacute exposure (four weeks) and 17 mg/kg/day following subchronic exposure (15 weeks). Teal and Evans (1982) observed 50% lethality in mice after 31 days (the mice received an average daily dose of 44 mg/kg intraperitoneally for 22 days followed by a ten-day observation period).

2. Short-Term Effects

Sub-lethal doses of acrylamide produce a number of adverse health effects in experimental animals and humans. Many of these effects reflect the neurotoxic properties of acrylamide on the peripheral or central nervous system. As noted earlier, acrylamide toxicity is cumulative, and thus duration of exposure is an important consideration. Summarized below are several representative studies involving short-term exposure (1 to 30 days). Studies involving longer exposures are summarized in the following section.

Agrawal et al. (1981a) studied the effect of a single oral dose of acrylamide on neurotransmitter levels in the brain. Six-week-old male Fischer rats were administered a single dose of 0, 25, 50 or 100 mg/kg by gavage (six to eight animals per dose). The rats were killed 24 hours later and specific brain regions were removed for analysis. Significantly increased binding of ^{3}H-spiroperidol was observed in striatal tissue at all doses of acrylamide ($P<0.05$). In animals that received 100 mg/kg, there was significantly increased binding of ^{3}H-strychnine in medulla and of serotonin in frontal cortex ($P<0.05$). No changes were observed in muscarinic receptors in striatum, benzodiazepine receptors in frontal cortex or GABA receptors in cerebellum.

Miller et al. (1983) studied the effect of single doses of acrylamide on retrograde axonal transport in rats. Animals were dosed with acrylamide by intraperitoneal injections (five animals per dose). Retrograde transport was assessed by movement of ^{125}I-labeled nerve growth factor (injected into front foot pads) toward spinal ganglia. Acrylamide doses of 25 mg/kg or higher resulted in significant inhibition of retrograde transport, but doses of 15 mg/kg or less did not. Multiple injections of 15 mg/kg/day caused inhibition of transport after five doses (cumulative dose of 75 mg/kg). Hind limb foot-splay was not affected until ten doses (cumulative dose of 150 mg/kg) had been administered, indicating that retrograde transport is a more sensitive indicator of acrylamide-induced neurotoxicity than the foot-splay test. The results of this study suggest that a single dose of 15 mg/kg is a NOAEL.

Prineas (1969) carried out light and electron microscopic studies of nervous system tissue from cats treated with acrylamide. Acrylamide (10 mg/kg) was administered daily by subcutaneous injection to five cats (2.5 to 4 kg). One cat was killed on day 11 of treatment. No neurologic signs (ataxia, hind limb weakness) had developed at this time, but electron microscopic examination of the tibial nerve revealed occasional fibers with increased number of neurofilaments and enlarged or swollen mitochondria. In the other cats, neurologic signs developed between days 17 and 22 of treatment. These cats were killed between days 22 and 49. Microscopic examination of intramuscular and tibial nerves from one cat killed on day 22 showed fragmented axons and myelin-ovoid formations. In animals examined after 32 or more days of treatment, sections of upper cervical spinal cord had degenerating myelin sheaths in the spinocerebellar tracts, in the gracile tracts and in the white matter adjacent to the anterior fissure. Electron microscopy revealed increased numbers of neurofilaments in occasional fibers in the tibial nerve, and in a majority of large diameter fibers by day 22 of treatment. Myelin degeneration was usually evident by 49 days. In all animals treated for 32 days, cell bodies in the dorsal root ganglia had some loss of the normal parallel arrangement of granular endoplasmic reticulum, breakdown of polyribosomes, release of ribosomes into the cytoplasm and an increase in the cytoplasm of electron dense material. Similar changes were observed in the anterior horn cells.

Peripheral neurotoxicity of acrylamide in dogs was assessed by Thomann et al. (1974). Six- to 12-month-old beagles were given acrylamide orally in gelatin capsules at daily doses of 5 or 15 mg/kg. Three male and three female dogs received 5 mg/kg/day (seven days/week) for 60 days. After three weeks of treatment, they became inactive, displaying sedation, ataxia and muscular weakness which was particularly noticeable in the jaw muscles. After 60 days of treatment, three of six dogs developed occasional swelling and fragmentation of myelin sheaths and axons, especially sciatic nerve distal branches. Additional effects observed in five male and five female dogs treated with 15 mg/kg/day acrylamide for 22 days included dilated pupils, conjunctivitis, salivation, difficulty in breathing, muscular stiffness of hind legs and convulsions. One female dog in the 15 mg/kg/day group died two days after the end of acrylamide treatment. Light microscopic examination of the peripheral nerves (sciatic, fibular, tibial) from one of the surviving animals in this group revealed swelling of the myelin sheaths and fragmentation of axons. The distal branches of the sciatic nerve appeared to be more severely involved than the proximal parts.

Gorzinski et al. (1979) studied the effect of acrylamide on a number of parameters (both neural and non-neural) in male and female CDF Fischer-344 rats (254 to 288 g). Acrylamide monomer was administered in drinking water at levels of 0, 1, 3, 10 or 30 mg/kg day for 21 consecutive days. Five rats/sex constituted each dose group. The animals were observed daily for overt signs of toxicity. Food and water consumption and body weight were recorded weekly. The onset of neuropathy was tested by the landing foot-spread method of Edwards and Parker (1977). At termination, clinical chemistry determinations of serum urea nitrogen (BUN), alkaline phosphatase (AP) and glutamic pyruvic transaminase (GPT) activities were conducted for all animals. All animals were subjected to gross necropsy with organ and body weights and organ-to-body weight ratios determined. Histological examination was done on brain, spinal cord and peripheral nerve (brachial plexus, femoral and sciatic nerves) from all controls and high dose animals and selected animals from the other dose groups. Sections of peripheral nerve from three animals/sex/dose were fixed for electron microscopy, but only tissue for the control and highest dose groups was processed for examination.

Some of the animals receiving 30 mg/kg/day began to exhibit an increase in hind limb splaying by the end of the second week of treatment. Midway through the third week, there was a definite trend apparent in this group, and a slight decrease in body weight gain was observed for both sexes. Food consumption decreased significantly in females during the third week, and water consumption also decreased. Males receiving 30 mg/kg/day had increased GPT and decreased AP activities at termination. Also, some differences in organ-to-body-weight ratios were observed in the animals receiving 30 mg/kg/day. Treatment-related effects were also noted in the peripheral nerves, but not in brain and spinal cord, both at the light and electron microscopic levels. No statistically significant effects were observed in animals receiving lower doses of acrylamide (1, 3 or 10 mg/kg/day) except that one male rat treated at 10 mg/kg/day exhibited some abnormalities in peripheral nerve at the light microscopic level of observation. The investigators determined that the dose levels of 1 and 3 mg/kg/day for 21 days could be interpreted as NOAELs in both sexes.

A number of cases of human exposure to acrylamide have been described in the literature. A review of this information (NIOSH 1976) indicates that exposure to acrylamide produces a consistent toxicity profile in humans which depends on the duration of exposure. Early signs and symptoms of acrylamide poisoning in humans are tenderness in fingers, numbness in lower limbs, excessive perspiration of the limbs, desquamation of the hands and muscular weakness of hands and feet. Prolonged exposure causes loss of body weight, apathy and hypersomnolence, as well as emotional disturbance, positive Romberg's sign, loss of proprioceptive sensations (vibration and position), weakened tendon reflexes and various motor difficulties (ataxic gait, foot drop, muscle atrophy). Retention of urine and feces also may occur. Neurological defects are noted in both the peripheral and central nervous systems. Most cases of human exposure to acrylamide occur under occupational circumstances. Exposure is generally through the skin or lungs, and quantitative estimates of exposure dosages are rarely available.

Igisu et al. (1975) described a case in which a family of five (three adults and two children) were exposed to acrylamide for about one month through ingestion and external use of well water contaminated with 400 mg/L of acrylamide. The symptoms which developed in the adults were consistent with the general pattern described earlier. Assuming daily ingestion of 2 L of water and a body weight of 70 kg, it would appear that a dose of 11 mg/kg/day is toxic in adult humans. It is not possible to estimate a NOAEL in humans from this study.

3. Longer-Term Exposure

Effects following long-term exposure to acrylamide are generally the same as those that appear after shorter exposures, except that lower daily doses are required. No studies of lifetime exposure to acrylamide were located in the literature. Studies described below involved exposure regimens from about 30 days to one year.

The effect of administration of acrylamide by intravenous, intraperitoneal, intramuscular, oral or subcutaneous routes was studied in cats by Kuperman (1958). Daily doses of 1, 2, 5, 10, 15, 25, 40 or 50 mg/kg were administered. Groups of 3 to 11 cats (strain, sex and age unspecified) were used at each dose. Effects of chronic poisoning, as measured by the development of ataxia, appeared at roughly identical total doses and after equivalent latent periods, irrespective of the route of administration. The length of the latent period from start of the treatment was inversely related to the dose and varied between 125 days (with the 1 mg/kg/day dose) and 2 days (with the 50 mg/kg/day dose). Clinical signs appeared when the animals had received an average of 102 mg/kg total dose over a period of a few days to four months. The continued daily administration of acrylamide after the appearance of postural, motor and behavioral disturbances resulted in the development of profound weakness and culminated in death. The average cumulative dose required for the moribund stage of poisoning was 320 mg/kg (about three times the cumulative dose required to produce ataxia). Necropsy of cats did not reveal any characteristic morphological or microscopic pathology of brain or spinal cord. Microscopic examination of other organs, urinalysis, blood chemistry and liver function tests also were negative. Administration of a single dose of 65 mg/kg, or repeated administration of 15 to 40 mg/kg produced characteristic electroencephalographic (EEG) changes, including a pronounced increase in wave frequency, complete abolition of large voltage slow waves and desynchronization from all areas of the cerebral cortex.

The effect of feeding acrylamide mixed with polyacrylamide polymer (polymer by itself is essentially non-toxic) in the diet was studied in adult Dow-Wistar rats (McCollister et al. 1964). Groups of ten male and ten female eight-week-old rats were maintained on diets which resulted in daily ingestion of 0, 0.3, 0.9, 3, 7, 9, 11, 30 or 40 mg/kg acrylamide monomer. At the end of the experiments, the animals were killed and necropsied. After 90 days, animals receiving 0.3, 0.9 or 3 mg/kg/day exhibited no abnormality in gross appearance, behavior, food consumption, growth nor gross and microscopic appearance of tissues. Similarly, rats maintained on 7, 9 or 11 mg/kg/day acrylamide for 189 days did not exhibit any toxic effects, except for one male rat which developed hindquarter ataxia after receiving 9 mg/kg/day for 56 days. In the groups maintained on a diet delivering 30.0 mg/kg/day of acrylamide, one animal demonstrated loss of control of hindquarters after 21 days, and five others were affected with the same condition within 90 days. By 42 days, all of the males in the 30 mg/kg/day groups were dead with only four of ten females surviving. These were taken off the acrylamide diet and completely recovered by 57 days on the control diet. In the animals receiving 40 mg/kg/day acrylamide, loss of hind quarter function was observed after 14 days of dosing, with complete loss of control in most rats by 24 days. On the 25th day, the animals in this group were put on a control diet. Recovery was gradual, but complete by 55 days on the control diet.

A one-year feeding study of cats (strain unspecified) receiving various concentrations of acrylamide in the diet was reported by McCollister et al. (1964). The diet, fed *ad libitum*, delivered calculated daily doses of 0.03, 0.1, 0.3, 1.0, 3.0 or 10.0 mg/kg. Cats were fed acrylamide five days/week (average daily doses of 0.02, 0.07, 0.2, 0.7, 2.1 or 7.1 mg/kg) and observed for weight, gross appearance and behavior. Two animals per dose and three controls were used. Two of the three controls died within the first month, however. The third was terminated after 171 days. For comparison purposes another cat was added as a control at terminal autopsy. At a daily dose of 7.1 mg/kg, the cats developed weakness in their hind quarters by 26 days (total dose 200 mg/kg); by 40 days, loss of control of hind legs was observed; and by 52 days (total dose of 370 mg/kg), loss of control of all four legs was observed. At this time one of the cats was killed and necropsied; the other was put on an acrylamide-free diet. The latter recovered completely within 53 days. Cats fed on the diet delivering an average dose of 2.1 mg/kg/day developed hindquarter trembling at 26 days and hindquarter weakness by 68 days. This weakness persisted during a dosing period of 299 additional days. One of the two cats given a dose of 0.7 mg/kg developed signs of weakness in the hind legs by 240 days of dosing. One of the animals dosed at 0.2 mg/kg/day died at 106 days, apparently for reasons unrelated to the acrylamide treatment. The other cat exhibited no adverse reaction throughout the year. The four animals at the two lowest dose levels (0.02 and 0.07 mg/kg/day) died of spontaneous diseases before 102 days. Light microscopic examination of the brain, spinal cord and sciatic nerve revealed no evidence of adverse effects of acrylamide ingestion at any of the doses, including the 10 mg/kg/day dose. A NOAEL of 0.2 mg/kg is indicated by the results of this study. However, the reliability of this study is limited by the small numbers of animals employed (two per dose) and the high incidence of deaths (presumably from causes other than acrylamide exposure).

McCollister et al. (1964) also studied the effects of acrylamide on female monkeys (strain and age unspecified) exposed for about one year (349 to 363 days). One monkey was used per dose of 0.03, 0.1, 1, 3, or 10 mg/kg/day; two monkeys were given 0.3 mg/kg/day and two monkeys served as controls. The 10 and 3 mg/kg doses were given intragastrically, and lower doses were administered in bananas. Animals received acrylamide 5 days/week (average daily doses of 0.02, 0.07, 0.2, 0.7, 2.1 or 7.1 mg/kg). Monkeys maintained on 0.02, 0.07, 0.2 or 0.7 mg/kg acrylamide/day exhibited no evidence of adverse effects as judged by growth, appearance, behavior, periodic neurologic examination, terminal hematologic (no details given) examination, or gross and microscopic study (at necropsy) of liver, kidney, brain and spinal cord tissues. The monkey that received 2.1 mg acrylamide/kg/day sometimes exhibited knee jerk and pupillary reflexes that were more sluggish than in controls, but this was considered to be insignificant by the authors. The monkey maintained on the acrylamide dose of 7.1 mg/kg/day exhibited some weakness of the hind quarters at 29 doses (cumulative dose of 290 mg/kg), and extreme weakness after 49 doses (cumulative dose of 490 mg/kg). This animal was returned to an acrylamide-free diet on day 70, and complete recovery was observed 54 days later. These results indicated to the authors that doses up to 0.7 mg/kg/day produced no toxic effects, but that doses of 2.1 or 7.1 mg/kg/day produced definite signs of neurotoxicity. The no-effect level of acrylamide in the monkey, as suggested in this study, is about 0.7 mg/kg.

Fullerton and Barnes (1966) subjected six- to eight-week-old male Porton strain rats to a daily intake of acrylamide in the diet for up to 48 weeks. The diet contained 0, 100, 200, 300 or 400 ppm acrylamide, which resulted in daily intakes of 6 to 9 mg/kg (100 ppm), 10 to 14 mg/kg (200 ppm), 15 to 18 mg/kg (300 ppm) or 20 to 30 mg/kg (400 ppm). Electrophysiological and histological studies were carried out. Conduction velocity in the nerve fibers supplying the small muscles on the plantar surface of the hind paws in ten control rats was 56 meters/second (±5.8 SD) compared with 44 meters/second (±2.2 SD) in 11/15 rats treated at 200 ppm (10 to 14 mg/kg) for six months, or 400 ppm (20 to 30 mg/kg) for two to three months. Animals treated at 400 ppm (20 to 30 mg/kg) had neurologic abnormalities of the hind limbs at about three weeks, that became severe at eight weeks. At 300 ppm, rats (15 to 18 mg/kg) developed slight abnormalities at four weeks, becoming severe at 10 weeks. At the 200 ppm dose (10 to 14 mg/kg), slight leg weakness appeared by 12 weeks, and by 16 weeks the abnormality had become severe. An additional 30 weeks on this diet did not lead to greater effects. At 100 ppm (6 to 9 mg/kg) the rats began to develop slight leg weakness after 40 weeks on the diet; this weakness did not intensify over the remaining eight weeks of the experiment. Histopathologic findings included extensive degeneration of peripheral nerves, with distal parts more affected than proximal segments. Macroscopic findings at necropsy included distended urinary bladders and wasting of hind limb musculature. Nerve conduction velocity in motor fibers to the muscles of the hind paw was reduced significantly in severely affected animals. When measured again following recovery from severe paralysis, mean conduction velocity had returned to the normal level.

Hopkins (1970) investigated the effect of acrylamide in five female and two male baboons (Papio hamadryas) weighing 9.4 to 15.4 kg. Acrylamide was administered in oranges or bananas as a 10% aqueous solution. One of the baboons may not have eaten all the fruit into which acrylamide was injected. This was reflected in the delay in onset of signs of toxicity when compared with the other animals receiving the same dose. Of the remaining six baboons, one was treated with 20 mg/kg/day for 29 days, another with 15 mg/kg/day for 94 days and four others with 10 mg/kg/day over a period of 89 to 192 days. Lack of coordination and weakness of the hind quarters was first observed on the 16th day in the animal receiving 20 mg/kg/day, on the 42nd day in the animal receiving 15 mg/kg/day, and between 42 and 97 days in the animals receiving 10 mg/kg/day. Recovery began 2 to 12 days after cessation of treatment. Wallerian-type degeneration of the peripheral nerves from baboons given 10 mg/kg/day for 118 days and biopsied 19 days later was evident by light microscopic study. Microscopic examination of both motor and sensory nerves revealed marked reduction in the number of myelinated fibers.

Burek et al. (1980) evaluated the subchronic toxicity of acrylamide in CDF rats. Acrylamide was administered in drinking water daily for 90 days to ten female rats and 23 to 29 male rats at each dose level (0, 0.05, 0.2, 1, 5, or 20 mg/kg/day). Ten males per group were held for 144 days after the treatment period for recovery studies. Additional rats were included for interim sacrifice and ultrastructural evaluations during the study.

The onset of neuropathy was evaluated by the limb-splaying method. Hematological parameters measured at day 76 of exposure (in seven of ten animals from both the control and 20 mg/kg group) and at the termination of exposure (all groups) included: packed cell volume (PCV), total red cell count (RBC), total and differential white cell count (WBC) and hemoglobin concentration (Hgb). Urinalyses were performed on day 76 in controls only. Attempts to do so in the 20 mg/kg animals were unsuccessful. Urinalyses were performed in all groups at the termination of the exposure period. Standard clinical chemical analysis of blood was done at the end of the exposure period in all groups and in four males per dose group during the recovery period. Parameters measured were: blood urea nitrogen (BUN), alkaline phosphatase (AP), serum glutamine pyruvic transaminase (SGPT) and serum cholinesterase (ChE).

A number of toxic signs were observed in the animals exposed at 20 mg/kg/day. Both sexes had a marked decrease in body weight gain, becoming significant by day 13 in the males and by day 20 in the females. A decrease in water consumption also was observed so that, by the end of the exposure period, the difference was statistically significant ($P<0.05$) in the females and marginal (but not significant) in the males. Absolute body weights also were decreased in the 20 mg/kg/day dose group, as were organ weights for brain, liver, thymus, kidney and testis. Heart weight was significantly decreased in females, with a trend toward a decrease seen in the males. Organ-to-body weight ratios for heart, liver, brain and kidney were increased in both sexes receiving 20 mg/kg/day. A significant decrease in the organ-to-body weight ratio was reported for the thymus (females only) and testes.

Serum ChE was significantly decreased at sacrifice (93 days) in the females receiving 20 mg/kg/day. AP activity was increased (statistically significant, $P<0.05$) in these females at that time, but the investigators suggested that this change may not have been treatment-related. Values for PCV, RBC and Hgb levels were all decreased in the animals receiving 20 mg/kg/day on day 76 and at sacrifice.

In the group receiving 20 mg/kg/day, animals of both sexes had statistically significant increases in the landing foot-spread measurement by day 22. This became even more pronounced at day 29, at which time the test was discontinued so that the animals would not be injured. Peripheral nerve and spinal cord lesions were observed by light and electron microscopy in all rats given the 20 mg/kg dose. Electron microscopic observations made at 33 days of treatment in the males revealed myelin and axonal degeneration with focal loss of axons. At sacrifice, all females exhibited severe degeneration in the sciatic nerve (50% or more of all fibers affected). This degeneration was characterized by severe demyelinization, axonal degeneration and loss of axons. Males exhibited the same changes, half of them (5/10) at the severe level (>50% of the neurons affected), the other half at the moderate level (20% to 50% of the neurons affected). In addition, degenerative myelopathy was observed in the fasciculus gracilis of the spinal cord. This damage was observed in at least one of the three sections made through the cord (cervical, thoracic and lumbar levels) of 5/10 males and 9/10 females. The changes were characterized by demyelinization and swollen astrocytes and axons. The severity varied from equivocal to slight. No treatment-related lesions were observed at the light microscopic level in sections of the brain from the cerebrum, cerebellum or midbrain.

Other effects which were judged to be treatment-related in the animals treated with 20 mg/kg/day included skeletal muscle atrophy, especially of the hind limb (2/10 males, 8/10 females), slightly increased hematogenous pigmentation of the spleen (4/9 females), hyperkeratosis and ulcerative gastritis in the non-glandular stomach (4/10 males), atrophy of mesenteric fat (8/10 females), testicular atrophy (10/10 males), focal or multifocal mineralization in the seminiferous tubules (5/10 males), increased cellular debris and/or decreased spermatogonic elements in the tubular lumina of the epididymis (9/10 males), vacuolization of the smooth muscle of the wall of the urinary bladder (1/10 males, 2/9 females) and pulmonary inflammation (3/10 males, 5/10 females).

In animals treated with 5 mg/kg/day, no significant changes were observed at the end of the exposure period (92 or 93 days) in body weight gain, absolute body weight and relative and/or absolute organ weights, except for the liver in the males. Both the absolute organ weight and the liver-to-body weight ratio were increased significantly. No effects were observed on water consumption, the landing foot-spread test or blood enzyme levels. Significant decreases in PCV, RBC and Hgb levels were observed in the females. Treatment-related effects in the peripheral nerves were observed histologically at the light microscopic level at this dose. The effects were of the same type as described for the highest dose, but they were generally less severe, varying from equivocal to very slight (focal or multifocal changes in individual neurons) in 9/10 males and 6/10 females. Ultrastructural alterations also were similar to those observed at the 20 mg/kg/day dose, although of a lesser degree of severity. Whereas about 55% of the fields tabulated in the 20 mg/kg group were affected, only about 34% of the fields were affected in the 5 mg/kg group.

No significant changes occurred in any non-neurological parameters measured in rats treated with the three lower doses (0.05, 0.2 or 1 mg/kg/day). No peripheral nerve lesions could be visualized at the light microscopic level. However, upon examination with the electron microscope, animals dosed at 1 mg/kg/day exhibited axolemmal invaginations in approximately 25% of the fields examined (about twice the control level). No significant alterations were observed at the two lowest dose levels. Therefore, on the basis of the most sensitive measure of toxicity (electron microscopic examination of peripheral nerves) in this study, a NOAEL of 0.2 mg/kg/day is estimated for CDF rats.

Schaumburg et al. (1982) examined whether electrophysiologic changes preceded axonal degeneration in acrylamide-treated monkeys. Monkeys were injected daily with doses of 1, 2, 3 or 10 mg/kg of acrylamide. The earliest abnormality was detected at one to two weeks in animals dosed at 10 mg/kg. A latency shift in the onset of near-field brain stem somatosensory evoked potential (SEP) activity overlying the gracile nucleus was noted following unilateral electrical stimulation of the peripheral nerve. The SEP configuration gradually returned to normal after cessation of acrylamide treatment. Animals dosed at 1 mg/kg of acrylamide developed SEP abnormality only after 18 months of daily dosing. Non-invasive brainstem SEP thus provided a sensitive test for early detection of peripheral neuropathy of the distal-axonopathy type.

Pryor et al. (1983) described neurobehavioral effects of acrylamide in adult male Fischer-344 rats. Acrylamide was administered orally five days/week for 15 weeks at doses of 0, 6.6, 9.6, 13.8 or 19.9 mg/kg (average daily doses of 0, 4.7, 6.9, 9.9 or 14.2 mg/kg). Toxicity was assessed at regular intervals by a battery of tests designed to detect toxicant-induced alterations in neuromotor function (grip strength), coordination (rotation orientation) and motor activity. Responsiveness to painful stimuli was assessed using a standard tail flick analgesic assay. Visual, auditory and proprioceptive functions were tested using a multisensory conditioned pole-climb avoidance procedure. Body weight and rectal temperature were recorded concurrently with the above tests. All rats dosed with 14.2 mg/kg/day died between week 7 and 15. The highest dose of acrylamide that was not lethal was 9.9 mg/kg/day. After 15 weeks of treatment at 9.9 mg/kg/day, a significant reduction in weight gain was observed. Recovery occurred within three weeks after cessation of dosing. Forelimb grip strength was affected significantly at this dose after 12 and 15 weeks of treatment. Recovery was complete three weeks after cessation of dosing. Hind limb grip strength was affected in a dose-related manner, being statistically significant in the groups receiving 9.9 and 6.9 mg/kg/day. These rats became progressively weaker and were slow to recover.

At the end of 15 weeks of dosing, the average hind limb grip strength of rats treated with 9.9 mg/kg/day of acrylamide was 27.3% of controls; six weeks after the end of dosing, this value was 73.8% of controls. The 9.9 mg/kg/day dose of acrylamide markedly impaired rotation orientation after 15 weeks of dosing, but recovery was rapid and complete three weeks after cessation of dosing. A significant difference in the acoustic startle effect was observed after 15 weeks of dosing at 9.9 mg/kg/day, and a greater difference in the reaction to the air-puff stimulus was observed. Startle to the air puff stimulus was also significant in the 6.9 mg/kg dosing group. Responses by treated rats to both stimuli were different than responses by control rats throughout the recovery phase. Only the 9.9 mg/kg/day dose of acrylamide impaired responses to all three stimuli of multi-sensory conditioned avoidance response (CAR). The CAR performance was still impaired markedly six weeks after cessation of dosing. At the 4.7 mg/kg/day concentration no significant effects were observed. There were no significant effects on motor activity, thermal sensitivity or rectal temperature at any dose level. Based upon the observation of several neurobehavioral parameters, a NOAEL of 4.7 mg/kg/day is estimated in rats.

B. Quantification of Non-Carcinogenic Effects

1. One-Day Health Advisory

No study was located in the literature which provides dose-response data sufficient to establish a NOAEL following a single oral exposure to acrylamide. The study of Miller et al. (1983) established a single dose NOAEL of 15 mg/kg, using an extremely sensitive index of acrylamide action (rate of axonal transport). However, the route of administration in this study was intraperitoneal injection. It is preferable to base an oral risk factor calculation on a study involving oral exposure, but in the case of acrylamide this deviation seems justifiable. The results presented in Section III. indicate that acrylamide is rapidly and completely absorbed from the gastrointestinal tract and that metabolism, distribution and clearance of acrylamide are similar following either oral or parenteral administration. Moreover, several studies have demonstrated that the toxicity of acrylamide is not significantly different when given orally or parenterally (Kuperman 1958, Edwards and Parker 1977, Shiraishi 1978). For these reasons it is considered acceptable to employ the NOAEL established by Miller et al. (1983) in the calculation of the one-day HA.

The one-day HA for the 10-kg child is calculated as follows:

One-day Health Advisory for Child

$$\frac{(15\ \text{mg/kg})(10\ \text{kg})}{(1\ \text{L})(100)} = 1.5\ \text{mg/L}$$

where:

15 mg/kg = NOAEL following acute exposure in rats based on no decrease in retrograde axonal transport in sensory neurons (Miller et al. 1983).

10 kg = assumed weight of child.

1 L = assumed volume of drinking water consumed in one day by a 10-kg child.

100 = uncertainty factor. This uncertainty factor was selected based upon the NAS/ODW guidelines in which a NOAEL from an animal study is used.

It is assumed that 100% of an oral dose is absorbed. Therefore, fractional absorption from the gastrointestinal tract is not included in the calculation.

The one-day HA for the 70-kg adult is calculated as follows:

One-day Health Advisory for Adult:

$$\frac{(15\ \text{mg/kg})(70\ \text{kg})}{(2\ \text{L})(100)} = 5.25\ \text{mg/L}$$

where:

15 mg/kg = NOAEL following acute exposure of rats based on no decrease in retrograde axonal transport in sensory neurons (Miller et al. 1983).

70 kg = assumed weight of adult.

2 L = assumed volume of drinking water consumed in one day by a 70-kg adult.

100 = uncertainty factor. This uncertainty factor was selected based upon NAS/ODW guidelines in which a NOAEL from an animal study is used.

2. Ten-Day Health Advisory

Few dose-response data are available from which a ten-day HA can be derived. The only study providing a NOAEL following multiple exposures over a period roughly equal to ten days is that by Gorzinski et al. (1979). These investigators administered acrylamide orally (in drinking water) to rats and observed effects for 21 days. On the basis of the hind foot-splaying method, analysis of blood enzymes and microscopic analysis of nerve tissue, a NOAEL of 3 mg/kg/day was identified. Since the NOAEL is based upon an oral exposure study, gastrointestinal absorption does not need to be included as a factor in the calculation.

The ten-day HA values for the 10-kg child and the 70-kg adult are calculated as follows:

Ten-Day Health Advisory for Child:

$$\frac{(3\ \text{mg/kg/day})(10\ \text{kg})}{(1\ \text{L/day})(100)} = 0.3\ \text{mg/L}$$

where:

3 mg/kg/day = NOAEL following 21-day exposure of rats based on an absence of functional or histologic evidence of nerve damage in rats (Gorzinski et al. 1979).

10 kg = assumed weight of child.

1 L/day = assumed drinking water consumption by a 10-kg child.

100 = Uncertainty factor. This uncertainty factor was selected based upon NAS/ODW guidelines in which a NOAEL from an animal study is used.

Ten-Day Health Advisory for Adult:

$$\frac{(3\ \text{mg/kg/day})(70\ \text{kg})}{(2\ \text{L/day})(100)} = 1.05\ \text{mg/L}$$

where:

3 mg/kg/day = NOAEL following 21-day exposure of rats based on an absence of functional or histologic evidence of nerve damage in rats (Gorzinski et al. 1979).

70 kg = assumed weight of adult.

2 L/day = assumed drinking water consumption of a 70-kg adult.

100 = uncertainty factor. This uncertainty factor was selected based upon NAS/ODW guidelines in which a NOAEL from an animal study is used.

An alternate approach to calculation of the 10-day HA values is to divide the one-day values by a factor of 10. This results in the following HA values:

10-kg child: $\frac{1.5\ \text{mg/L}}{10} = 0.15\ \text{mg/L}$

70-kg child: $\frac{5.25\ \text{mg/L}}{10} = 0.53\ \text{mg/L}$

The Office of Drinking Water feels it is more appropriate to exercise the first option in this case. This is because the calculation is based upon actual toxicological data (even if for 21 days rather than ten days), rather than judgmental extrapolation from a study of shorter duration (single dose).

3. Adjusted Acceptable Daily Intake

No dose-response data from studies of lifetime exposure to acrylamide are available which establish a NOAEL suitable for calculation of the ADI. However, several studies have been reported which estimate or identify NOAELs following periods of exposure from 90 days to one year.

McCollister et al. (1964) fed acrylamide mixed with polyacrylamide in the diet to adult Dow-Wistar rats for 90 days. Groups of ten male and ten female animals received daily doses equivalent to 0, 0.3, 0.9 or 3.0 mg/kg monomer. Gross appearance, food consumption, growth and gross and microscopic examination revealed no adverse effects at any dose. Thus, 3.0 mg/kg may not necessarily be the highest NOAEL under the conditions of this particular experiment.

Burek et al. (1980) evaluated the toxicity of acrylamide in rats following subchronic (90 day) exposure in drinking water. Animals were administered daily doses of 0, 0.05, 0.2, 1, 5 or 20 mg/kg. The parameters measured were gross neuropathy (as observed by the limb-splaying method) ultrastructure of peripheral motor nerves, hematology, clinical chemistry and body and organ weights. On the basis of the most sensitive measure of toxicity (the ultra-structural examination of motor nerves) it was concluded that 0.2 mg/kg was the NOAEL.

Pryor et al. (1983) described the neurobehavioral toxicity of acrylamide in adult male Fischer 344 rats following subchronic oral exposure (five days/week for 15 weeks). The dosed animals were subjected at regular intervals to a number of behavioral tests. Body weight gain and rectal temperatures also were monitored. No significant changes were observed in any of the parameters measured at the lowest dose (4.7 mg/kg/day). Thus, a NOAEL of 4.7 mg/kg/day was determined for neurobehavioral end-points in adult rats.

McCollister et al. (1964) studied the effects of acrylamide in monkeys treated for up to one year. Single animals constituted the dose "groups" at the following dose levels: 0.02, 0.07, 0.2, 0.7, 2.1 or 7.1 mg/kg/day when averaged over a 7-day week. Two monkeys constituted the dose "groups" that received daily doses of 0 or 0.2 mg/kg. Animals were dosed five days/week (intragastrically at the two highest doses, in bananas at the other concentrations). Monkeys treated at doses up to and including 0.7 mg/kg received 255 doses over 363 days. The authors reported that no adverse effects were noted in animals receiving doses of 0.7 mg/kg or less. The monkey dosed at 2.1 mg/kg/day sometimes showed sluggish knee jerk and pupillary reflexes, but the authors considered this to be insignificant. In light of evidence gathered in subsequent, more sophisticated experimentation, these observations could be considered significant. Therefore, it is concluded that, under the conditions of this experiment, a NOAEL of 0.7 mg/kg/day can be identified.

McCollister et al. (1964) also studied the effects of long-term exposure to acrylamide in cats. Three animals served as untreated controls; two animals per group received doses of acrylamide at average daily levels of 0.02, 0.07, 0.2, 0.7, 2.1 or 7.1 mg/kg/day in the diet, five days/week for approximately one year. Animals fed doses of 0.7 mg/kg and higher exhibited signs of neuropathy, although this was not reflected morphologically upon light microscopic examination. Under the conditions of the study, the dose of 0.2 mg/kg was determined to be a NOAEL. The usefulness of this study is limited by the small number of animals used (two per dose) and a high incidence of death due to causes unrelated to acrylamide exposure.

Of these five longer-term studies which identify NOAELs, the Burek study is judged to be the best one to use for the derivation of an ADI and an AADI for acrylamide. The reason for this is that in the Burek study a large number of parameters were measured, including electron microscopic examination of neurons. This provided a more sensitive means of evaluating the neuropathic effects of acrylamide than was employed in either the Pryor study or the three McCollister studies.

The ADI for a 70-kg adult is calculated as follows:

ADI:

$$\frac{(0.2\ \text{mg/kg/day})(70\ \text{kg})}{1{,}000} = 0.014\ \text{mg/day}$$

where:

0.2 mg/kg/day = NOAEL based upon lack of significant incidence of neuropathic lesions observed following ultrastructural examination of peripheral motor nerve in the rat (Burek et al. 1980).

70 kg = assumed weight of adult.

1,000 = uncertainty factor. This uncertainty factor was selected based, in part, upon NAS/ODW guidelines in which a NOAEL from an animal study is used. A further consideration was that the NOAEL from a less than lifetime exposure (90-day study) was used and that evidence exists that acrylamide toxicity is based upon total cumulative dose.

Using 0.014 mg/day as the ADI, and assuming the 70-kg adult consumes two liters of drinking water per day, the AADI is calculated as follows:

AADI:

$$\frac{0.014\ \text{mg/day}}{2\ \text{L/day}} = 0.007\ \text{mg/L}$$

This AADI calculation assumes 100% of the human exposure derives from drinking water. This value may be modified upon the availability of relative source contribution data providing human exposure estimates from food, air and possibly the occupational environment.

For ease of comparison, Table VIII-1 summarizes the NOAEL values and corresponding ADI and AADI values from each of the five studies considered for this calculation.

C. Carcinogenic Effects

Several reports indicate that acrylamide may be a carcinogen. Bull et al. (1984) investigated the effect of acrylamide on the incidence of lung adenomas in male and female A/J mice following oral and intraperitoneal routes of administration. Oral doses of 6.25, 12.5 or 25.0 mg/kg acrylamide (>99% pure) dissolved in distilled water, three times/week, for eight weeks (average daily doses of 2.7, 5.4 or 10.7 mg/kg/day, respectively) produced an increase in the yield of lung adenomas in both males and females in a dose-dependent manner ($P<0.01$). Acrylamide administered by the intraperitoneal route also resulted in a dose-dependent increase in lung adenomas ($P<0.01$). In these studies, oral exposure was significantly more effective in promoting carcinogenesis than intraperitoneal injection or topical application.

In another study groups of male and female Fisher 344 rats received drinking water containing acrylamide monomer at 0, 0.01, 0.1, 0.5 or 2.0 mg/kg/day for up to two years (Johnson, et al. 1984). After a year, significant depression of body weight was observed in the highest dose males. Distal neuropathy was observed in the peripheral nerves of animals in this group. Tumor incidence was not increased significantly in the groups receiving 0.01 or 0.1 mg/kg/day. Male rats receiving 0.5 mg/kg/day had significantly increased incidences of scrotal mesothelioma. Statistically significant increased incidences of

Table VIII-1 Comparison of Values from Candidate Studies Identifying Non-Carcinogenic End-Points of Toxicity

Reference	Species	Duration	NOAEL (mg/kg/day)	ADI (mg/day)	AADI (mg/L)
Burek et al. 1980	Rat	90 days	0.2	0.014	0.007
McCollister et al. 1964	Rat	90 days	3	0.21	0.105
Pryor et al. 1983	Rat	15 weeks	4.7	0.33	0.165
McCollister et al. 1964	Monkey	1 year	0.7	0.05	0.025
McCollister et al. 1964	Cat	1 year	0.2	0.014	0.007

tumors in the following tissues were determined in rats treated at 20 mg/kg/day: Females - mammary glands (benign and malignant), central nervous system (malignant), thyroid gland follicular epithelium (benign and malignant), mouth (benign), uterus (malignant) and cliotoral gland (benign); Males - scrotal mesothelioma (malignant) and thyroid gland follicular epithelium (benign).

D. Quantification of Carcinogenic Risk

The evidence from Bull et al. (1984) and the Johnson et al. (1984) studies in mice and rats show that acrylamide has significant carcinogenic potential.

On the basis of the results observed from the rat drinking water study (Johnson et al. 1984), EPA Carcinogen Assessment Group (CAG) has prepared a draft quantitative risk assessment of acrylamide exposure (USEPA 1985). In this draft assessment, CAG derived several carcinogenic potency factors from different sets of dose-response data. The CAG recommended, however, that the potency factor (q_1^*) of 3.7 $(mg/kg/day)^{-1}$ derived from the combination of tumor incidence data on mammary gland, thyroid and uterus in the females be used for estimating the increased lifetime risk of human exposure to acrylamide. Assuming that a 70-kg adult ingests two liters of water per day over a 70-year lifetime, the estimated excess cancer risks at 10^{-4}, 10^{-5} and 10^{-6} would be 1.0 µg/L, 0.1 µg/L and 0.01 µg/L, respectively. (These estimates were made by the Office of Drinking Water.)

E. Existing Guidelines and Standards

The present occupational standard for acrylamide of 0.3 mg/m^3, as a time-weighted-average concentration, was chosen on the basis of the Threshold Limit Value of 0.3 mg/m^3 recommended and adopted by the American Conference of Governmental Industrial Hygienists in 1967 (NIOSH 1976). The National Institute for Occupational Safety and Health recommended in 1976 that no employee be exposed to a concentration greater than 0.3 mg acrylamide per cubic meter of air determined as time-weighted-average concentration for up to a 10-hour work shift in a 40-hour work week (NIOSH 1976). Based on a joint report of the International Labor Office and the World Health Organization, it appears that no standards for acrylamide have been promulgated by countries other than the United States (NIOSH 1976).

F. Special Considerations

1. High Risk Subpopulations

No evidence was found that any particular subpopulation other than workers has increased risk of adverse effects following acrylamide exposure than the general population.

2. Interactions

A number of chemicals have been found to influence acrylamide toxicity. Diethyl maleate (DEM) increased acrylamide toxicity, presumably by acting to decrease cellular levels of glutathione. Vitamin B_6 supplementation provided a modest reduction in acrylamide-induced neuropathy by one test (but not by another). Several derivatives of acrylamide, which are themselves neurotoxic, increased neurotoxicity when administered with acrylamide, but it was not clear if the effects were additive or synergistic. Pretreatment of rats with chemicals (such as phenobarbital or DDT) which induce hepatic microsomal enzymes, resulted in increased acrylamide neurotoxicity in one study, but not in another.

G. Summary

The recommended values for the one-day and ten-day HAs for both adults and children, and the lifetime AADI are summarized in Table VIII-2.

Table VIII-2 Summary of Quantification of Toxicological Effects of Acrylamide

		Drinking water concentration (mg/L)		
		10-kg Child	70-kg Adult	Reference[a]
One-Day Health Advisory		1.5	5.25	Miller et al. 1983
Ten-Day Health Advisory		0.3	1.05	Gorzinski et al. 1979
AADI		--[b]	0.007	Burek et al. 1980
Excess Cancer Risk	10^{-4}	--[b]	1.0 μg/L	Johnson et al. 1984
	10^{-5}	--[b]	0.1 μg/L	
	10^{-6}	--[b]	0.01 μg/L	

[a] Reference for NOAEL used in calculating HA or AADI values.

[b] Not calculated because the child will not remain in the high risk population (i.e., 10-kg child) for a lifetime.

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